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An antibacterial composition of thienamycin-type compound and a dipeptidase inhibitor

Introduction

French Patent 2,272,684 discloses the addition of specific unsaturated fatty acids to antibiotics, for instance to penicillin, in order to increase the antibacterial effect against resistant bacteria.

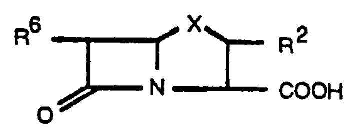
A new class of fused ring β-lactam antibiotics, including thienamycin and its semi-synthetic derivatives, epithienamycins, and olivanic acids, has recently been described. These compounds which will be defined more extensively below, are hereinafter referred to as the "thienamycin class of compounds". These compounds have a high level of antibacterial activity, but are subject to extensive metabolism by mammalian species.

The kidney was identified as the primary site of metabolism, and an enzyme was purified from renal extracts which catalyzed the inactivation of thienamycin by hydrolysis of the β-lactam. By such criteria as cytological localization, substrate specificity and susceptibility to enzyme inhibitors, this enzyme is very similar if not identical to a widely studied renal dipeptidase (E.C.3.4.13.11), also referred to in the literature as "dehydropeptidase-I". However, the β-lactamase activity is exhibited only toward the thienamycin class of compounds. Indeed, there exists no precedent example of the mammalian metabolism via β-lactam cleavage of any representative of the classical β-lactam antibiotics, the penicillins and cephalosporins.

Detailed Description of the Invention: Thienamycins

The term "thienamycin class of compounds" is used to identify 6- and (optionally) 2-substituted pen-2-em-3-carboxylic acids and 1-carbadethia-pen-2-em-3-carboxylic acids or 1-azabicyclo [3.2.0]hept-2-ene-7-one-2-carboxylic acids.

Specific compounds particularly useful in this invention are represented structurally in the following formula I:



I

wherein X can be CH_2 or S; R^2 can be hydrogen; $-S-CH_2CH_2NHR^3$, wherein R^3 is hydrogen, acetyl, formimidoyl, acetimidoyl; $-S(O)-CH=CHNHCOCH_3$ and $-S-CH=CHNHCOCH_3$; and R^6 is

$$-\underset{\substack{|\\ R^7}}{CH}CH_3$$

wherein R^7 is hydrogen, hydroxy or hydroxy-sulfonyloxy, or R^6 is H. All possible stereoisomeric forms are included within the above structural definition.

All of these compounds within Formula I are described in the literature. When X is CH_2, and R^2 is $SCH_2CH_2NH_2$, and R^6 is $CH(OH)CH_3$, the compound is known as thienamycin, an antibiotic produced by fermentation of *S. cattleya*, deposited as NRRL 8057 at Northern Regional Research Laboratories, U.S. Department of Agriculture, Peoria, Illinois, U.S.A., on November 18, 1974 described and claimed in U.S. Patent 3,950,357, issued April 13, 1976. The N-substituted derivatives of thienamycin, i.e., in the formula I above wherein R^3 is other than hydrogen, are disclosed and claimed in co-pending U.S. applications and their published foreign equivalents. The fermentation product N-acetyl thienamycin (R^6 is $CH(OH)CH_3$, and R^3 is acetyl), also called 924A, is claimed in Belgian Patent No. 848,346, issued May 16, 1977. The N-imidoyl derivatives are covered in Belgian Patent No. 848,545, issued May 20, 1977. The unsaturated side chain-containing compound, also called N-acetyl-dehydrothienamycin or $924A_5$ is a fermentation product of S. cattleya, NRRL 8057 claimed in U.S. Patent 4,162,323 issued July 24, 1979, and also in Belgian Patent No. 866,035, issued October 17, 1978. Epimeric forms of N-acetyl thienamycin, also called 890A, and $890A_3$, as well as the desacetyl 890A, and desacetyl $890A_3$ are disclosed, respectively in published French Patent 7634887 granted April 14, 1980 and Belgian Patent 848,349, issued May 16, 1977. Epimeric forms of the unsaturated thienamycin, also called $890A_2$ and $890A_5$ are claimed in published French Patent 7711891 granted April 20, 1977.

The 6-hydroxysulfonyloxyethyl-containing N-acetyl compounds, also called $890A_9$ or $890A_{10}$, are claimed respectively, in published French Patent 7,734,456, granted June 23, 1980 and published French Patent No. 7,734,457, granted March 3, 1980. Desacetyl analogues of $890A_9$ and $890A_{10}$ are claimed in DE—OS 28 05 701, and also in French Patent 7,803,666, granted May 5, 1980, and DE—OS 28 05 724, and also in French Appln. 7,803,667, filed February 9, 1978. Some of these latter compounds in the $890A_9$ and $890A_{10}$ series are also known as derivatives of olivanic acid (see Corbett

et al., *J. Chem. Soc. Chem. Commun.* 1977, No. 24, pp. 953-54). Compounds of the Formula I above when R^2 is hydrogen, also called descysteaminyl thienamycins, are claimed in French Patent 7814597 granted October 27, 1980, and also in Belgian Patent 867,227, issued November 20, 1978.

When R^6 is hydrogen, and X is CH_2, these compounds are disclosed in Belgian Patent 860,962, and in its published German equivalent Off. 2,751,624.1, filed November 18, 1977.

A thienamycin-type antibiotic in which R^2 is $-SCH_2CH_2NHAc$ and R^6 is C_2H_5, has been named PS—5 and is reported by K. Okaimura et al., *J. Antibiotics 31* p. 480 (1978), see also Belgian Patent 865,578.

The compounds in which X is S, also called "penems", are described by R.B. Woodward in "Recent Advances in the Chemistry of β-Lactam Antibiotics", J. Elks (Ed), The Chemical Society, London, 1977, p. 167; R.B. Woodward, Abstracts of Uppsala University 500 Years Symposium on Current Topics in Drug Research, Uppsala, Sweden, October 1921, 1977. *Acta. Pharm. Suecica,* Vol. 13, Supplement, p. 23, and U.S. Patent 4,070,477, issued January 24, 1978.

Particularly preferred members within the thienamycin class of compounds are the N-formimidoyl and N-acetamidoyl derivatives of thienamycin. The crystalline form of N-formimidoyl thienamycin, which has recently been described, is also useful in the practice of this invention. An example illustrating a preferred way of making this compound follows:

Illustrative Example

N-Formimidoyl thienamicyin, crystalline

Step A. Benzylformimidate hydrochloride

A 3 l. three-necked flask fitted with an addition funnel, overhead stirrer, and a reflux condenser, was charged with a mixture of benzyl alcohol (125 g., 1.15 mol) formamide (51 g., 1.12 mol) and anhydrous ether (1200 ml.). The mixture was stirred vigorously at room temperature (20—25°C) under a nitrogen atmosphere and benzoyl chloride (157 g., 1.12 mol) in 50 ml. of anhydrous ether was added dropwise using the addition funnel. The addition required approximately 50 minutes. .

The reaction mixture was stirred an additional 60 minutes at room temperature. The ether was removed by decantation and 300 ml. of acetic anhydride in 500 ml. of anhydrous ether was added. The mixture was stirred 30 minutes at room temperature. The precipitate was allowed to settle and the ether-acetic anhydride was again removed by decantation. The solid was collected by filtration, washed with 500 ml. of ether and dried *in vacuo* over KOH at 25°C for 2 hrs. to give 130 g. (67%) of benzylformimidate hydrochloride as a white solid.

The product was assayed by NMR δ (DMSO) 5.7 (s, 2H, ØCH$_2$), 7.5 (s, 5H, Ø), 9.0 (s, 1H, HC=N). The product is thermally unstable. It decomposes to formamide and benzyl chloride at 0°C and above. However, no appreciable decomposition was detected on storage at −20°C for 2 months.

Step B. Derivatization of Thienamycin

Thienamycin (in the form of a 6 l. aqueous solution, pH = 6.5, concentrate from the fermentation broth, containing 28 g. thienamycin) was placed in a large beaker (12 l) and cooled to 0°C. The beaker was equipped with a pH meter and an efficient high speed stirrer. The pH was raised to 8.5 by the careful addition of 3N KOH (KOH was added dropwise via syringe to the stirred solution). The solution was treated with 6 equivalents of solid benzyl formimidate hydrochloride (~100 g.) in portions while maintaining the pH at 8.5 ± 0.3 by the addition of 3N KOH (200 ml.) using a syringe. The addition required 3—5 min. The reaction mixture was stirred for 6 min. at 0°C and then assayed by liquid chromatography to insure completion of the reaction. The solution was adjusted to pH 7 with 1N HCl. The volume of the reaction mixture was measured, and the solution was assayed by UV. The neutralized reaction mixture was concentrated to 15 g./l. on the reverse osmosis unit at <10°C. The volume of the concentrate was measured and the pH was adjusted to 7.2—7.4, if necessary. The concentrate was filtered through a medium porosity sintered glass funnel to remove any solids present after concentration.

Step C. Dowex 50W × 2 Chromatography

The concentrate (750—1000 ml., 15—20 g.) was applied to 0°C. to a precooled 18 l. column of Dowex 50W × 2 in the potassium cycle (200—400 mesh resin) and the column was eluted at 0—5°C with distilled deionized water a flow rate of 90 ml/min. and a head pressure of 0—45 psig.

Forerun fractions of 4 l., 2 l., and one l., were collected followed by 18 fractions of 450 ml. each, and one final fraction of 2 l. Each fraction was assayed by UV (1/100 dilution, NH_2OH extinction was omitted) and the total amount of NFT present in each fraction was calculated. The beginning and end fractions were assayed for liquid chromatography purity and the desired rich cut fractions were combined. The pH of the combined rich cuts was determined by both pH meter and bromothymol blue indicating solutions and was adjusted to pH 7.2—7.4 if necessary. The combined rich cuts (3—4 l.) were then assayed by UV and the total formamidine content was determined, 15—16 g., 75% yield from the column. The rich cuts were concentrated on the reverse osmosis unit at <10°C as far as possible, then the concentration to 33 g./l. was completed on the circulatory evaporator at less than 28°C. A total volume of about 500 ml. concentrate was obtained.

Step D. Crystallization of N-Formimidoyl Thienamycin

The concentrate from the previous step is adjusted to pH 7.3, if necessary, and N-formimidoyl thienamycin content assayed by UV, was about 85—90%. The concentrate was filtered through a sintered glass funnel (medium porosity) into a large Erlenmeyer flask. Five volumes (~2200 ml.) of 3A ethanol was filtered into the concentrate and the solution was stirred at room temperature for 10 minutes and at 0°C for 12—24 hrs.

The crystals were filtered by suction filtration and washed with 0.1 volume (~250 ml.) of 0°C 80% 3A ethanol followed by 1/25 volume (100 ml.) of 3A ethanol at room temperature. The crystals were dried *in vacuo* for 12—24 hrs. to give approximately a 40% overall yield of N-formimidoyl thienamycin (10—12 g.).

Analytical results on a 50 g. blend of N-formimidoyl thienamycin, prepared as above, are as follows:

C, theory 45.42%; found, 45.82%

H, theory 6.03%; found, 5.72%

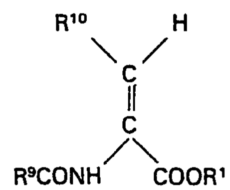
N, theory 13.24%; found, 13.10%

S, theory 10.10%; found, 10.14%

residue on ignition, predicted 0.5, found 0.47%, $[\alpha]_D^{25} = 89.4°$, T.G. = 6.8%, UV λ max 300 nm, E% =328.

Detailed Description of the Invention Dipeptidase Inhibitors

The chemical substances which selectively inhibit the metabolism of the dipeptidase [E.C.3.4.13.11], also called "dipeptidase inhibitors", include chemical compounds which are Z-2-acylamino-3-monosubstituted propenoates having the following formula



II

wherein R^9 and R^{10} are hydrocarbon radicals in the range respectively of 3—10 and 1—15 carbon atoms. In either of these hydrocarbon radicals R^9 and R^{10}, up to 6 hydrogens may be replaced by halogens, or a non-terminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter.

A terminal hydrogen in R^{10} can also be replaced by a hydroxyl or thiol group, which may be acylated, such as with an alkanoyl acid of 1—8 carbon atoms, or carbamoylated, including alkyl and dialkyl carbamate derivatives; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted alkyl amino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as a terminal amino acid grouping.

R^9 is preferably a branched alkyl or cycloalkyl radical (C_{3-10}), with a limitation that the carbon adjacent to the carbonyl cannot be tertiary. R^1 is hydrogen, loweralkyl (C_{1-6}) or dialkylamino-alkyl (e.g., $—CH_2CH_2N(C_2H_5)_2$, $—CH_2CH(CH_3)N(CH_3)_2$.

Some of the compounds with formula II above have asymmetric forms. Racemic Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic aid has been resolved. The activity resides in the dextrorotatory isomer, which has the S-configuration.

Within the definition of R^9, the following sub-groups are included:

$$—R^4 \qquad \text{II A}$$

wherein R^4 is a straight, branched or cyclic hydrocarbon radical of 3—10 carbon atoms which may be substituted as specified above in the definition of R^9;

$$—R^5R^{11} \qquad \text{II B}$$

wherein R^5 is cycloalkyl of 3—6 carbon atoms and R^{11} is either 1 or 2 alkyl substituents which may be

joined to form another ring on the cycloalkyl group, or R^5 and R^{11} may be substituted as specified above in the definition of R^9;

$$—R^{12}R^8 \qquad \text{II C}$$

wherein R^{12} is an alkylene group of 1—3 carbon atoms and R^8 is cycloalkyl of 3—6 carbon atoms which may be substituted as specified above in the definitions of R^9 and R^{10};

within these sub-groups, the following specific compounds are included:

II A: Z - 2 - isovaleramido - 2 - pentenoic acid; methyl Z - 2 - isovaleramido - 2 - butenoate; Z - 2-isovaleramido - 2 - butenoic acid; Z - 2 - benzamido - 2 - butenoic acid; Z - 2 - (3,5,5 - trimethylhexanamido) - 2 - butenoic acid; Z - 2 - cyclobutanecarboxamido - 2 - butenoic acid; Z - 2 - cyclopropanecarboxamido - 2- butenoic acid; Z - 2 - cyclopropanecarboxamido - 2 - pentenoic acid; Z - 2 - (3 - methylvaleramido) - 2- butenoic acid; Z - 2 - cycloheptanecarboxamido - 2 - butenoic acid; Z - 2 - nonamido-2 - butenoic acid; Z - 2 - cyclohexanecarboxamido - 2- butenoic acid; Z - 2 - (4 - methylvaleramido) - 2-butenoic acid; Z - 2 - t - butylacetamido - 2 - butenoic acid; Z - 2 - octanamido - 2 - butenoic acid; Z - 2-butyramido - 2 - butenoic acid; Z - 2 - valeramido - 2 - butenoic acid; Z - 2 - valeramido - 2 - pentenoic acid; Z - 2 - cyclopentanecarboxamido - 2 - butenoic acid; Z - 2 - (6 - methylheptanamido) - 2 - butenoic acid; Z - 2 - hexanamido - 2 - butenoic acid; Z - 2 - (3,7 - dimethyloctanamido) - 2- butenoic acid; Z - 2 - (3,7 - dimethyl - 6 - octenamido) - 2 - butenoic acid; Z - 2 - (5 - chlorovaleramido) - 2- butenoic acid; Z - 2 - (3 - chlorobenzoylamido) - 2 - butenoic acid; Z - 2 - (2 - chlorobenzamido) - 2- butenoic acid; Z - 2 - nonanamido - 2 - butenoic acid; Z - 2 - (6 - bromohexanamido) - 2 - butenoic acid; Z - 2-(3,3 - dimethylpropenamido) - 2 - butenoic acid; Z - 2 - benzamido - 2 - cinnamic acid; Z - 2 - benzamido-2 - pentenoic acid; Z - 2 - benzamido - 5 - methoxy - 2 - pentenoic acid; Z - 2 - benamido - 2 - hexenedioic acid; Z - 2 - isovaleramido - 2 - octenoic acid; Z - 2 - isovaleramido - 2 - cinnamic acid; Z - 2 - isovaleramido - 2 - hexenedioic acid; Z - 2 - cyclopropanecarboxamido - 2 - cinnamic acid; Z - 2 - cyclopropanecarboxamido - 2 - hexenedioic acid; Z - 2 - (5 - methoxy - 3 - methylvaleramido) - 2 - butenoic acid; Z - 2 - ethylthioacetamido - 2 - butenoic acid; Z - 2 - (2,2 - dichlorocyclopropane - carboxamido) - 2 - butenoic acid; Z - 2 - (2 - ethylhexanamido) - 2 - butenoic acid; Z - 2 - di - n - propyl - acetamido - 2 - butenoic acid;

II B: Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid; (+) - Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido)-2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - octenoic acid; Z - 2 - (2,2-dimethylcyclopropanecarboxamido) - 2 - hexenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido)-2 - cinnamic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5 - methoxy - 2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 4,4,4 - trifluoro - 2 - butenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 3 - (2 - chlorophenyl)propenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - hexenedioic acid; Z - 2 - (2 - ethylcyclopropanecarboxamido) - 2 - butenoic acid; Z-2 - (2,2 - diethylcyclopropanecarboxamido) - 2 - butenoic acid; Z - 2 - (2,2 - diethylcyclopropanecarboxamido) - 2 - pentenoic acid; Z - 2 - (2 - isopropyl - 2 - methylcyclopropanecarboxamido) - 2-butenoic acid; Z - 2 - (2 - methylcyclohexanecarboxamido) - 2 - butenoic acid; Z - 5 - cyano - 2 - (2,2-dimethylcyclopropanecarboxamido) - 2 - pentenoic acid; Z - 5 - (N,N - dimethyl - carbamoyl) - 2 - (2,2-dimethylcyclopropanecarboxamido) - 2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5-methanesulfonyl - 2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5 - ethoxycarbonyl - 2 - pentenoic acid; Z - 2 - (2 - methylcyclopropanecarboxamido) - 2- butenoic acid; methyl Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoate; ethyl Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoate; 2 - dimethylaminoethyl ester of Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid; 3 - diethylaminopropyl ester of Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - pentenoic acid; Z - 2 - (2,3 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid; Z - 2 - (3,3 - dimethylcyclobutanecarboxamido) - 2 - butenoic acid; Z - 2 - (2 - spirocyclopentanecarboxamido) - 2 - butenoic acid; Z - 2 - (2 - t - butyl - 3,3 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 4 - methyl - 2 - pentenoic acid; Z - 2-(2 - t - butylcyclopropanecarboxamido) - 2 - butenoic acid; Z - 2 - (2 - phenylcyclopropanecarboxamido)-2 - butenoic acid; Z - 3 - cyclohexyl - 2 - (2,2 - dimethylcyclopropanecarboxamido)propenoic acid; Z - 5-carboxy - 5 - (2,2 - dimethylcyclopropanecarboxamido) - 4 - pentenamidine; Z - 5 - dimethyl amino - 2-(2,2 - dimethylcyclopropanecarboxamido) - 2 - pentenoic acid; Z - 3 - cyclopropyl - 2 - (2,2 - dimethylcyclopropanecarboxamido)propenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2,5-hexadienoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 4 - phenyl - 2 - butenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 6 - mercapto - 2 - hexanoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5 - methylthio - 2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5 - phosphono - 2 - pentenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido)-2 - heptenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 5 - phenyl - 2 - pentenoic acid; Z-2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - nonenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - decenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - tri - decenoic acid; Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 6 - methoxy - 2 - hexenoic acid (and 5-

methoxy - 2 - pentenoic acid); Z - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 6 - methyl - 2 - heptenoic acid; Z - 4 - cyclohexyl - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - butenoic acid;

II C: Z - 2 - cyclobutylacetamido - 2 - butenoic acid; Z - 2 - cyclopentylacetamido - 2 - butenoic acid; Z - 2 - cyclohexylacetamido - 2 - butenoic acid; Z - 2 - (4 - cyclohexylbutyramido) - 2 - butenoic acid; Z - 2 - cyclopropylacetamido - 2 - butenoic acid; Z - 2 - cyclopropylacetamido - 2 - pentenoic acid; Z - 2 - (3 - cyclopentylpropionamido) - 2 - butenoic acid; Z - 2 - (3 - cyclohexylpropionamido) - 2-butenoic acid; Z - 2 - (4 - (2 - thienyl) - butyramido) - 2 - butenoic acid; Z - 2 - (4 - phenylbutyramido)-2 - butenoic acid; Z - 2 - (D,L - α - lipoamido) - 2 - pentenoic acid; Z - 2 - (D,L - α - lipoamido) - 2-cinnamic acid; Z - 2 - (3 - (2 - tetrahydrofuryl) - propionamido) - 2 - butenoic acid.

Particularly preferred substituents within the definition of R^9 above include the 2,2-dimethylcyclopropyl and the 2,2-dichlorocyclopropyl groups.

Within the definition of R^{10}, particularly preferred groups of compounds included N-alkyl (1—9 carbons) and N-methyl (1—9 carbons), having a terminal substituent which is a quaternary nitrogen, amine derivative, or amino acid derived group.

By the term "quaternary nitrogen" is meant a tetrasubstituted or heteroaromatic nitrogen which is positively charged. An ammonium moiety, substituted with hydrocarbon groups having 1—7 carbon atoms, which can be the same or different, is significative.

By the term "amino derivative" is meant a group such as amino, acylamino, ureido, amidino, guanidino and alkyl derivatives thereof.

By the term "amino acid derived group" is meant a moiety such as cysteinyl (—$SCH_2CH(NH_2)COOH$) or sarcosyl (—$N(CH_3)CH_2COOH$) in which a hydrogen joined to O, N or S of known amino acids is replaced.

Particularly preferred compounds from the most preferred groups of substitutents of R^9 and R are those wherein R^9 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl, and R^{10} is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent, or having a terminal substituent which is trimethylammonium, amidino, guanidino, 2-amino-2-carboxyethylthio, or ureido. Names of specific examples of these include:

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-amidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid;

Z-8-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid (racemic and dextrorotary forms); and Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid.

The Z configuration (J.E. Blackwood et al., *J. Am. Chem. Soc.*, *90*, p. 509 (1968)) is assigned to the above compounds on the basis of their NMR spectra by analogy with the work of A. Srinavasan et al. [Tetrahedron Lett., 891 (1976)].

Although these compounds of Formula II, when R^1 is H, are described and named as the free acids, it will be apparent to one skilled in the art that various pharmaceutically acceptable derivatives such as alkali and alkaline earth metal, ammonium, or amine salts, or the like can be employed as equivalents thereto. Salts such as the sodium, potassium, calcium, or tetramethylammonium salts are suitable.

Some of the compounds of Formula II are novel compounds which are claimed in published European Patent application 79 102 616.4, filed on July 24, 1979, and which do not form part of this invention.

In accordance with what is said above this invention is directed to an antibacterial composition characterized in that it contains a combination of a 6-substituted and optionally 2-substituted pen-2-em-3-carboxylic acid, a 1-carbadethia-pen-2-em-3-carboxylic acid or 1-azabicyclo[3.2.0]hept-2-ene-7-one-2-carboxylic acid and a dipeptidase (E.C.3.4.13.11) inhibitor, the weight ratio of the first named material to the dipeptidase inhibitor being within the range of 1:3 to 30:1.

Methods of Testing and Using the Invention

As noted, disposition studies with thienamycin, its natural analogs and its semi-synthetic derivatives have revealed a major metabolic degradation pathway of elimination in the various species examined (mouse, rat, dog, chimpanzee, Rhesus monkey). The extent of metabolism is reflected in low urinary recovery and short plasma half-lives. The nature of this degradation was demonstrated to be lactam cleavage by the renal dipeptidase (E.C.3.4.13.11), described first by Bergmann, M. and Schleich, H., *Z. Physiol. Chem.*, *205* 65 (1932); see also Greenstein, J. P. *Advances in Enzymology*, Vol. VIII, Wiley-Interscience, (1948), New York, and Campbell, B. J.; Lin, Y-C., Davis R. V. and Ballew, E., "The Purification and Properties of Particulate Renal Dipeptidase", *Biochim. Biophys. Acta.*, *118*, 371 (1966).

In order to demonstrate the ability of the compounds of Formula II to suppress the action of the renal dipeptidase enzyme, an *in vitro* screen procedure was followed. This measured the ability of compounds to inhibit hydrolysis of glycyldehydrophenylalanine (GDP) by a solubilized preparation of dipeptidase isolated from hog kidneys. The procedure is as follows: to a 1 ml. system containing 50 mM "MOPS" (3-(N-morpholino)propanesulfonic acid) buffer, pH 7.1, is added 5 μg of lyophilized enzyme, and the test compound at a final concentration of 0.1 mM. After a five minute incubation at 37°C, GDP is added to a final concentration of 0.05 mM. Incubation is continued for 10 minutes, at 37°C and hydrolysis of GDP is measured by the change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is expressed as the inhibitor binding constant, K_i. This is the concentration of the inhibitor which achieves 50% inhibition of enzyme.

The substrate GDP is employed in preference to thienamycin in this screen because it has a much higher maximal velocity of hydrolysis by renal dipeptidase, thereby reducing the amount of enzyme required. Both GDP and thienamycin have a similar affinity for renal dipeptidase; furthermore, K_i's of inhibitors tested have been identical for the two substrates.

In addition to this *in vitro* screen procedure, an *in vivo* screen was followed to measure the test compound's ability to inhibit metabolism as reflected by increase in urinary recovery of thienamycin from the mouse. The procedure involves co-administration of the test compound by the intravenous or subcutaneous route at a dose-rate of 10—100 mg/kg, with 10 mg/kg thienamycin. Thienamycin recovery in the urine over a 4 hour period is then compared with its recovery in a control group to which the test compound was not co-administered.

Urinary recovery of thienamycin was measured in all cases with the use of a cylinder or disc diffusion assay, conducted in a manner described in U.S. Patent 3,950,357. This bioassay, with *Staphylococcus aureus* ATCC 6538 as the test organism, has a useful response range from 0.04 μg/ml to 3.0 μg/ml.

The combination of the inhibitor and the thienamycin class compound can be in the form of a pharmaceutical composition containing the two compounds in a pharmaceutically acceptable carrier. The two can be employed in amounts so that the weight ratio of the thienamycin class compound to inhibitor is 1:3 to 30:1, and preferably 1:1 to 5:1.

The components can also be separately administered. For instance, the thienamycin class compound can be administered intramuscularly or intravenously in amounts of 1—100 mg/kg/day, preferably 1—20 mg/kg/day, or 1—5 mg/kg/day, in divided dosage forms, e.g., three or four times a day. The inhibitor can be separately administered, orally, intramuscularly, or IV, in amounts of 1—100 mg/kg/day, or preferably 1—30 mg/kg/day, or 1—5 mg/kg/day. The amounts of the two components administered during one day ideally are within the ratio limits denoted above.

The most preferred dosage levels presently known to applicants is as a single dose, of two crystalline compounds, one being N-formimidoyl thienamycin and the other being (+) Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, co-administered in a sterile aqueous IV injection form (sodium salt), at a level of 150 mg. of the thienamycin and either 75 or 150 mg of the octenoic acid. This dose is given to humans (each assumed to weigh about 80 kg.) from 1 to 4 times a day, or 2—8 mg/kg/day of the thienamycin class compound and 1—8 mg/kg-day of the inhibitor.

The components, whether adminstered separately or together are employed in pharmaceutically acceptable carriers such as conventional vehicles adapted for oral administration such as capsules, tablets, or liquid solutions or suspensions. The components separately or together, can also be dissolved in a vehicle adapted for administration by injection. Suitable formulations for oral use, may include diluents, granulating agents, preservatives, binders, flavoring agents, and coating agents. An example of an oral use composition is the combination of active ingredients, or the acid component alone, intermixed in the dry pulverulent state with gelatin, starch, magnesium stearate, and alginic acid, and pressed into a tablet.

As noted above, the presently known preferred method is parenteral administration of the thienamycin class compound and either co-parenteral administration or oral administration of the inhibitor compound.

Examples which illustrate this invention follow.

SECTION 1. EXAMPLES ILLUSTRATING ACTIVITY

Example 1

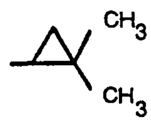
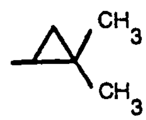
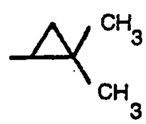
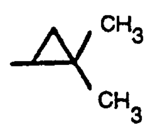
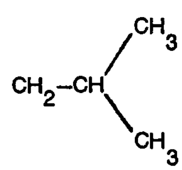
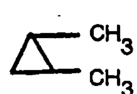
In Vitro Test Data

A 1 ml. system of 50 mM "MOPS" buffer, pH 7.1, is used. To this is added 5 μg of the pig renal enzyme and an amount of the test compound to bring its final concentration to 0.1 mM. After a five minute incubation at 37°C, an amount of GDP is added to bring its final concentration to 0.05 mM. The system is again incubated for 10 minutes, at 37°C. Hydrolysis of GDP is measured by its change in optical density with time at 275 nm. Inhibition of the enzyme is gauged by comparison to a standard run containing no inhibitor and is presented as percent inhibition. The K_i is a constant indicating the concentration of inhibitor necessary to produce 50% inhibition of enzyme. It is a calculated value obtained from running multiple *in vitro* assays, as above, at concentrations resulting in inhibition below

and above the 50% inhibition point. The results are presented in Table I.

TABLE I

Compounds $R^{10}-\overset{H}{C}=\overset{COOH}{C}-NHCOR^9$

Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
1	CH_2CH_3		98	0.18
2*	CH_3		98	0.39
2a*	CH_3		100	0.12
2b*	CH_3			19.8
3	CH_3		92	1.7
4	CH_2CH_3		87	3.2
5	CH_3	$-CH_2CH(CH_3)-CH_2C(CH_3)_3$	81	4.4
6	CH_3		83	4.6

*Compounds 2, 2a, and 2b are the racemic, dextrorotatory and levorotatory forms respectively.

TABLE I (Continued)

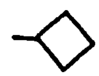
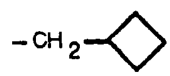
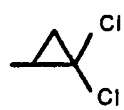
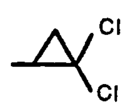
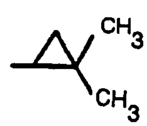
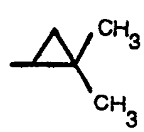
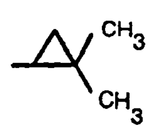
Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
7	CH_3	$-CH_2-CH(CH_3)_2$	91	6
8	CH_3		80	6.2
9	CH_3		83	6.6
10	CH_3		97	9
11	CH_3	$-CH_2-CH(CH_3)-CH_2CH_3$	82	10
12	$-(CH_2)_4CH_2$			.03
13	$-(CH_2)_5N^+(CH_3)_3$			
14	$-(CH_2)_5N^+(CH_3)_3$			1.11
15	$-(CH_2)_5-NH-C(CH_3)=NH$			
16	$-(CH_2)_5-NH-C(=NH)-N^+(CH_3)_2$			

TABLE I (Continued)

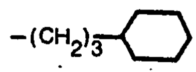
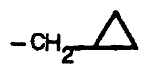
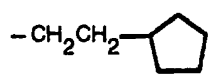
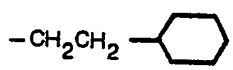
Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
17	CH_3	$-CH_2C(CH_3)_3$	75	20
18	CH_3	$-(CH_2)_6CH_3$	72	26
19	CH_3	$-(CH_2)_2CH_3$	69	30
20	CH_3		68	30
21	CH_3		64	22
22	CH_3	$(CH_2)_3CH_3$	64	32
23	CH_3		59	30
24	CH_3	$-(CH_2)_4CH(CH_3)_2$	57	
25	CH_3		56	
26	CH_3		54	
27	CH_3	$-CH_2-(CH_2)_3CH_3$	54	39
28	CH_3	$-(CH_2)_5CH_3$	49	
29	CH_3	$-CH(CH_2CH_3)CH_2CH_2CH_2CH_3$	33	
30	CH_3	$-CH(CH_2CH_2CH_3)_2$	13	
31	CH_3	$-CH(CH_3)_2$	31	
32	$HOO-CH_2CH_2$		90	5
33	CH_3	$-CH_2-CH(CH_3)-CH_2CH_2OCH_3$	88	9

TABLE I (Continued)

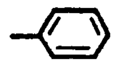
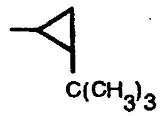
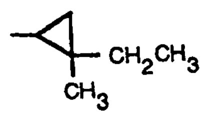
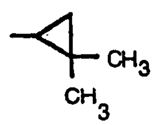
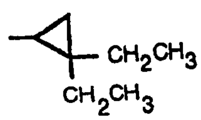
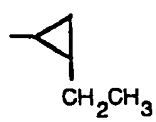
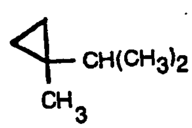
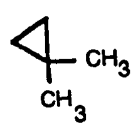
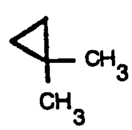
Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
34	CH_3	$CH_2CH_2CH_2CH_2CH_2Br$	70	19
35	CH_3	$CH_2CH_2CH_2CH_2Cl$	64	20
36	CH_3	$CH_2CH_2CH_2$ 	72	11
37	CH_3		90	6.5
38	$CH_3(CH_2)_4$	$CH_2-CH(CH_3)_2$	95	2.6
39	CH_3		100	0.45
40	$(CH_3)_2CH$		98	0.54
41	CH_3		98	0.86
42	CH_3		96	1.6
43	CH_3		95	3
44	CH_3CH_2		98	0.18
45	Ph		100	0.62

TABLE I (Continued)

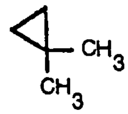
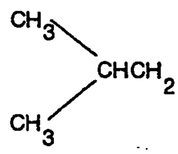
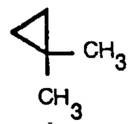
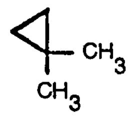
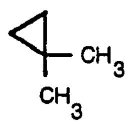
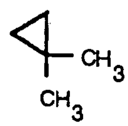
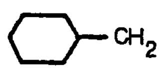
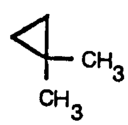
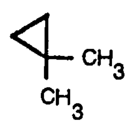
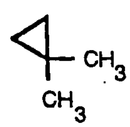
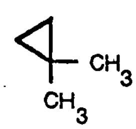
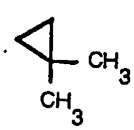
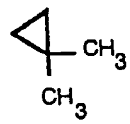
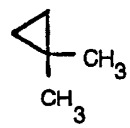
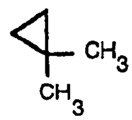
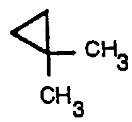
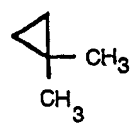
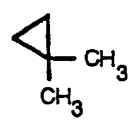
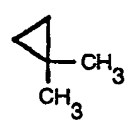
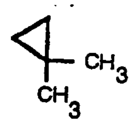
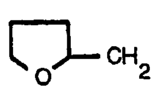
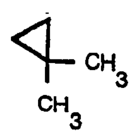
Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
46	$CH_3CH_2CH_2$		98	0.11
47			97	0.23
48	$CH_3(CH_2)_3$		100	0.11
49	$CH_3(CH_2)_4$		100	0.17
50	$HOOCCH_2CH_2$		98	0.145
51			100	0.15
52	$PhCH_2CH_2$		96	0.33
53	$CH_3SCH_2CH_2$		99	0.12
54	$CH_3SO_2CH_2CH_2$		96	0.5
55	$CH_3(CH_2)_5$		98	0.149

TABLE I (Continued)

Dipeptidase Inhibitor	R^{10}	R^9	% Inhibition at 10^{-4} M	K_i (μM)
56	$CH_3(CH_2)_6$		99	0.092
57	$CH_3(CH_2)_9$		96	0.14
58	$PhCH_2$		98	0.44
59	$CH_3O(CH_2)_3$			0.28
60	$CH_3OCH_2CH_2$		98	0.32
61	$(CH_3)_3CCH_2$			0.34
62	$(CH_3)_2CHCH_2CH_2$		98	0.15
63	$H_2OC(CH_2)_3$		99	0.048
64				0.39

Example 2

In Vivo Test Data

An *in vivo* assay on the mouse was conducted as follows: 20 g Charles River CD, female mice were injected subcutaneously with the chosen dose of the chemical inhibitor. About two minutes later, the dose of thienamycin was given intravenously. A control of thienamycin above was also conducted. The level of thienamycin in the urine as a % of the dose was measured using a bioassay technique. Results are found in Table II. The two test compound numbers are those from Table I. Compound 7 is 2-isovaleramido-2-butenoic acid; compound 10 is Z-2-cyclopropylcarboxamido-2-butenoic acid.

TABLE II

Compound	Dose, mg/kg Compound	Dose, mg/kg Thienamycin	% Urinary Recovery of Thienamycin
7	50	10	53
7	10	10	63
10	50	10	56
Control	–	10	25–30

Example 3

The compounds 2-isovaleramido-2-butenoic acid, Compound 7, and Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, Compound 2, were studied, in more detail *in vivo* in combination with thienamycin (THM), in the mouse. The general test procedure was similar to that of Example 2. Results are summarized in Table III and Table IV.

Example 4

In another mouse study, the systemic anti-bacterial activity of thienamycin was enhanced approximately three-fold by coadministering 2-isovaleramido-2-butenoic acid, see Table V.

TABLE V

Effect of Co-administered 2-Isovaleramido-2-butanoic acid on the Systemic Efficacy of Thienamycin on the Treatment of *Staphylococcus aureus* Infections

		ED_{50}, mg/kg
THM	Alone	0.2
	+ 100 mg/kg inhibitor	0.06

TABLE III

Effect of Co-administered 2-Isovaleramidobutenoic Acid (Compound 7) on the Urinary Recovery of Thienamycin in the Mouse [(a)]

Route [(b)] Compound 7	THM	mg/kg Dose Compound 7	THM	Urinary Recovery of THM, %
–	IV or SC	–	10	30 ± 5
SC	SC	0.3	10	33
SC	IV	2	10	42
SC	SC	2	10	47
SC	IV	10	10	53
SC	SC	50	10	54
SC	IV	50	10	53
SC	SC	80	10	59
SC	SC	100	10	81

(a) 20 g Charles River, CD_1 female mice.

(b) Co-administered.

TABLE IV

Effect of Co-administered Z-2-(2,2-Dimethylcyclopropane-carboxamido)-butenoic acid (Compound 2) on Urinary Recovery of Thienamycin in the Mouse [(a)]

Route [(b)] Compound 2	THM	mg/kg Dose Compound %	THM	Urinary Recovery THM, %
–	SC	–	10	30 ± 5
SC	SC	0.1	10	35
SC	SC	0.3	10	40
SC	SC	1	10	46
SC	SC	10	10	60
SC	SC	10	10	73

(a) 20 g Charles River, CD_1 female mice.

(b) Co-administered.

Example 5

A male beagle was used for a study of the effect of dipeptidase inhibitors on the urinary recovery of N-formimidoyl thienamycin. In a control study, the dog was given 5 mg/kg IV of the N-formimidoyl thienamycin without inhibitor. A second experiment used the same amount of N-formimidoyl-thienamycin, but also administered Z-2-isovaleramido-2-butenoic acid in 3 doses, each providing 20 mg/kg of the compound. The first dose was administered just after injection of the N-formimidoyl-thienamycin, the second at 40 min. and the third at 60 min. The third study employed a single dose (2 mg/kg) of Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-butenoic acid, administered just before injection of the N-formimidoyl thienamycin. The results are in Table VI.

TABLE VI

Urinary Recovery 3 Hours following the Administration of N-formimidoylthienamycin (5 mg/kg IV) in a Male Beagle

Test Compound	% Urinary Recovery
N-formimidoyl thienamycin	7.8
plus Z-2-isovaleramido-2-butenoic acid	46
plus Z-2-(2,2-dimethylcyclopropane carboxamido)-2-butenoic acid	53

SECTION 2. EXAMPLES ILLUSTRATING CHEMICAL PREPARATIONS

The inhibitor compounds are novel compounds claimed in a copending application. These compounds are made by condensing directly the appropriate 2-keto acid and amide:

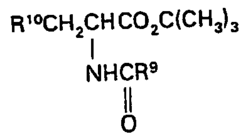
$$\underset{\text{III}}{R^{10}CH_2\overset{\overset{O}{\|}}{C}CO_2H} \; + \; \underset{\text{IV}}{R^9\overset{\overset{O}{\|}}{C}NH_2} \; \rightarrow \; \text{II}$$

wherein R^9 and R^{10} are as defined. The general reaction conditions involve mixing approximately 1—4:1 parts of the acid to the amide in an inert solvent such as toluene or methyl isovalerate and heating at reflux with azeotropic removal of water for from 3—48 hours, preferably 5—24 hours. The solution when cooled normally yields the product in crystalline form, but the product can also be isolated using a base extraction process. The product can be recrystallized by using generally known techniques. An optional modification of this procedure requires an additional small amount of p-toluenesulfonic acid as catalyst during the reaction.

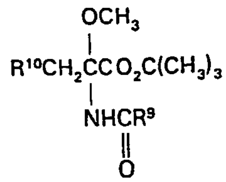
Another route to the novel inhibitor compounds uses an α-amino acid t-butyl ester in reaction with an acid chloride.

$$\underset{\text{V}}{R^9\text{—}\overset{\overset{O}{\|}}{C}Cl} \; + \; \underset{\text{VI}}{R^{10}\text{—}CH_2\text{—}\underset{\underset{NH_2}{|}}{\overset{H}{C}}\text{—}COO\text{—}C(CH_3)_3}$$

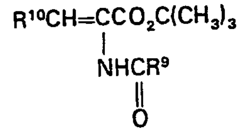
This reaction takes place in the presence of base, such as triethylamine, in a solvent such as methylene chloride. The resulting N-acylated product (VII) is then oxidized by treatment with t-butyl hypochlorite followed by addition of sodium methoxide. This yields the 2-methoxy derivative (VIII) and/or its elimination product, the α,β-unsaturated ester (IX). Further treatment with anhydrous hydrochloric acid converts either VIII or IX (or the mixture of both) to the desired α,β-unsaturated free acid (II).



VII



VIII



IX

Some compounds wherein R^{10} has a terminal substituent which is an amino, quaternary nitrogen, thiol or carboxyl, derivative can be made most conveniently from an intermediate having a bromo substituent instead of the desired terminal substituent, followed by condensation with the desired thiol, amino, acid, or the like.

More detail about preparation of the compounds is found in the following examples.

Example 6
Z-2-Isovaleramido-2-butenoic Acid

A solution of 1.07 g (10.5 mmole) of 2-keto-butyric acid and 0.71 g (7.0 mmole) of isovaleramide in 15 ml of toluene was stirred under reflux with collection of H_2O in a small Dean-Stark trap. After 5 hrs, the solution was cooled, resulting in fairly heavy crystallization. After standing, the solid was collected on a filter and washed with toluene and then with CH_2Cl_2. Yield of white crystals = 0.47 g, mp 172—174° (slight prelim. softening). The material was recrystallized from diisopropyl ketone. Tlc (4:1 toluene-AcOH) now showed only a faint trace of the other isomer. Yield of white crystals = 0.32 g (25%), mp 175° (slight prelim. softening). NMR indicated essentially exclusively Z-isomer.

Anal. ($C_9H_{15}NO_3$)		Calcd.	Found
	C	58.36	58.59
	H	8.16	8.55
	N	7.56	7.43

Example 7
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-pentenoic acid

A solution of 1.74 g (15 mmole) of 2-keto-valeric acid and 1.13 g (10 mmole) of 2,2-dimethylcyclopropanecarboxamide in 20 ml of toluene was refluxed with stirring with collection of H_2O in a small Dean-Stark trap. After 20 hrs. the solution was cooled and treated with a gentle stream of N_2. Before much of the solvent had evaporated, crystallization was induced by scratching. After standing, the solid was collected on a filter and washed with toluene and some Et_2O. Yield of white crystals = 0.63 g (30%), mp 154.5—155.5° (slight prelim. softening). Tlc (4:1 toluene-AcOH) showed only an extremely faint trace of the other isomer. NMR was consistent with the Z-configuration.

Anal. ($C_{11}H_{17}NO_3$)		Calcd.	Found
	C	62.53	62.86
	H	8.11	8.27
	N	6.63	6.75

Example 8
Z-2-(3-Cyclopentylpropionamido)-2-butenoic acid

A solution of 1.41 g (10 mmole) of 3-cyclopentylpropionamide and 1.53 g (15 mmole) of 2-keto-butyric acid was stirred and refluxed under a small Deak-Stark trap. After 8 hrs. the solution was cooled, resulting in heavy crystallization. The solid was collected on a filter and washed with toluene and CH_2Cl_2. Yield of white crystals = 1.44 g, mp 180.5—182° (prelim. softening). The material was recrystallized from methyl ethyl ketone. Yield of white needles = 0.63 g (28%), mp 184—185° (slight prelim. softening). Tlc (4:1 toluene-AcOH) now showed a single spot, and NMR indicated essentially pure Z-isomer.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.97	63.99
H	8.50	8.67
N	6.22	6.27

Example 9

Z-2-(2-Ethylhexanamido)-2-butenoic acid

10 g. of 2-ethylhexanoyl chloride was added dropwise with stirring to 25 ml of cold conc. NH_4OH solution, resulting in immediate precipitation. The mixture was allowed to stir for 2 hrs., then filtered, and air dried to give 6.5 g. of amide. 1.4 g (10 mmole) of the above compound and 1.5 g of ketobutyric acid (15 mmole) were refluxed in 25 ml toluene for 15 hrs. with removal of water. The reaction mixture was cooled and partly evaporated with a stream of N_2. Crystallization of product occurred after standing for 3 hrs. The crystals were collected, washed 3x with toluene, and air dried. There were isolated 1.13 g (50%) of product, mp 160—162°. NMR was in accord with the assigned structure and indicated <5% E isomer. Tlc (4:1 toluene-AcOH) showed a single spot.

Anal. ($C_{12}H_{21}NO_3$)	Calcd.	Found
C	63.40	63.63
H	9.30	9.43
N	6.16	5.88

Example 10

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-butenoic acid

1.53 g (15 mmoles) of 2-ketobutyric acid, 1.13 g (10 mmoles) of 2,2-dimethylcyclopropanecarboxamide and 20 ml of toluene stirred at reflux for 10 hours. After cooling the crystalline solid was filtered and washed with toluene (3 x 10 ml) and dried to give 1.06 g of product, mp 140—141 °C. Tlc (4:1 toluene-AcOH) showed essentially one spot and the NMR spectrum fit the desired structure.

Recrystallization from EtOAc gave after drying 0.533 g of product mp 142—143.5°, homogenous by tlc.

Anal. ($C_{10}H_{15}NO_3$)	Calcd.	Found
C	60.90	60.92
H	7.67	7.71
N	7.10	7.38

Example 11

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenedioic acid

A mixture of 1.0 g of 2,2-dimethylcyclopropanecarboxamide, 2.4 g. of 2-ketoadipic acid and 25 ml. of methyl isovalerate was heated under reflux for 4 hrs, with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). After standing at room temperature overnight, the crystalline precipitate was filtered, washed with ether and recrystallized from ethyl acetate to give 0.23 g. of product, m.p. 163—165°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{17}NO_5$)	Calcd.	Found
C	56.46	56.20
H	6.71	6.83
N	5.49	5.32

Example 12

Z-2-(2,2-Diethylcyclopropanecarboxamido)-2-butenoic acid

A mixture of 2.3 g of 2-ketobutyric acid, 2.0 g of 2,2-diethylcyclopropanecarboxamide, and 25 ml of toluene was heated under reflux for 16 hours with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). No product precipitated upon cooling. Ether (25 ml) was added and

the mixture was extracted with saturated $NaHCO_3$ (3 times). The combined extracts were acidified with concentrated HCl. The gummy precipitate crystallized when triturated with water. Recrystallization from ethyl acetate gave 0.31 g of product, m.p. 129—30°. The NMR spectrum was consistent with the desired structure.

Anal. ($C_{12}H_{19}NO_3$)	Calcd.	Found
C	63.98	64.01
H	8.50	8.62
N	6.22	6.21

Example 13

2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexanoic acid

Step A: DL-Norleucine t-butyl ester

General procedure of R. Roeske, *J. Org. Chem. 28*, p. 1251 (1963).

To a suspension of 9.82 g (75 mmole) of DL-norleucine in 80 ml of dioxane in a 500 ml. pressure bottle cooled in an ice bath was added slowly (with swirling) 9 ml of concentrated H_2SO_4. The resulting mixture was cooled in a dry ice bath as 80 ml of liquid isobutylene was added. The mixture was allowed to warm to room temperature and shaken under autogenous pressure for ~ 23 hours. After most of the isobutylene had been vented off, the slightly hazy solution was cooled in ice and then added to a cold-mixture of 400 ml of 1*N* NaOH and 500 ml of Et_2O. After shaking in a separate funnel, the layers were separated, and the aqueous fraction was washed with an additional 100 ml of Et_2O). The Et_2O solution was shaken with 150 ml of 0.5 *N* HCl. The acidic aqueous fraction was treated with 2.5 *N* NaOH until strongly basic and then shaken with 250 ml of Et_2O. The Et_2O solution was dried ($MgSO_4$), filtered, and concentrated on the rotovac. After prolonged pumping on high vacuum over a steam bath, final yield of clear, colorless residual oil = 9.04 g (65%). NMR now showed only a trace of dioxane. TLC (9:1 $CHCl_3$—MeOH) showed a single spot.

Step B: N-(2,2-Dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester

To a solution of 8.98 g (48 mmole of DL-norleucine t-butyl ester and 5.05 g (50 mmole) of triethylamine in 100 ml of CH_2Cl_2 stirredin an ice bath under a drying tube was added dropwise (over a period of 75 min.) a solution of 6.39 g (48 mmole) of 2,2-dimethylcyclopropanecarbonyl chloride (M. Elliot and N.R. James, British Patent No. 1,260,847 (1972)) in 50 ml of CH_2Cl_2. Precipitation of Et_3N HCl occurred during the addition, especially toward the end. As the ice gradually melted, the mixture was allowed to warm to room temperature. After 16 hrs., the mixture was shaken with 200 ml of 0.5 *N* HCl. The CH_2Cl_2 fraction was washed with an additional 200 ml of 0.5 N HCl, then with 2 x 200 ml of 0.5 *N* NaOH, and finally 200 ml of H_2O. The CH_2Cl_2 fraction was dried with $MgSO_4$, treated with charcoal, and filtered through Celite. The filtrate was concentrated on the rotovac (finally under high vacuum). Yield of light orange residual oil = 11.93 g (88%). Tlc (2:1 hexane-EtOAc) showed a single spot. NMR and IR were in accord with the assigned structure. After standing for several days, the unused portion of this material crystallized: m.p. 52—>65°C.

Step C: t-Butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate

Based on procedure of H. Poisel and V. Schmidt, *Chem. Ber., 108* p. 2547 (1975).

To a solution of 6.37 g (22.5 mmole) of N-(2,2-dimethylcyclopropanecarbonyl)-DL-norleucine t-butyl ester in 35 ml of Et_2O stirred at room temperature under N_2 in the dark was added 2.69 ml (2.45 g, 22.5 mmole) of t-butyl hypochlorite. After 15 min., a solution of sodium methoxide prepared by dissolving 0.52 g (22.6 mmole) of sodium in 35 ml of MeOH was added. Stirring was continued at ambient temperature under N_2 in the dark. After 16.5 hrs., the precipitated NaCl was filtered off. The filtrate was diluted with Et_2O and washed successively with 3 x 50 ml of 0.5 *N* HCl, 50 ml of saturated Na_2CO_3, and 2 x 50 ml of H_2O. The E_2O phase was dried over $MgSO_4$ and filtered. The filtrate was concentrated on the rotovac. The pale, golden-yellow residual oil (6.45 g) was subjected to preparative high pressure liquid chromatography, resulting in the separation and isolation of 273 mg and 496 mg of the two diastereomers of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexanoate (respective mp's 114—118° and 124—125.5°) as well as 1.97 of a single isomer (apparently Z) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate (colourless oil).

Step D: 2-(2,2-Dimethylcyclopropanecarboxamido)-2-hexenoic acid

A solution of 0.84 g (3.0 mmole) of t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-hexenoate in 10 ml of Et_2O saturated with anhydrous HCL was allowed to stand at room temperature under a drying tube. After 17 hrs., the solution was evaporated, and the residual gum was dissolved in 10 ml of saturated $NaHCO_3$. This solution was washed with an additional 15 ml of 0.5 *N* HCl, then dried ($MgSO_4$), filtered, and concentrated to give a viscous oil. The oil was crystallized from toluene. Yield of

white crystals = 0.32 g (47%), m.p. 119—122°. TLC (4:1 toluene-AcOH) showed a single spot. NMR indicated essentially pure Z-isomer (*Note*: Treatment of the methanol adduct, t-butyl 2-(2,2-dimethylcyclopropanecarboxamido)-2-methoxyhexenoate, with anhydrous HCl in Et_2O under similar conditions gave the same product.)

Example 14

(+)-Z-2-(2,2-Dimethylcyclopropanecarbonylamino)-2-octenoic acid, sodium salt

The reagents, (+)-2,2-dimethylcyclopropanecarboxamide, 7.0 g.; 2-keto-octanoic acid ethyl ester, 14.7 g.; 50 mg. of p-toluene sulfonic acid; and 100 ml. of toluene were charged to a 250 ml. three-necked flask under a Dean Stark trap containing several molecular sieve pellets. The mixture was refluxed vigorously for 27 hours. The resultant light yellow solution was cooled and concentrated *in vacuo*, at a water bath temperature of 45°C., in the presence of water to help remove toluene. The gummy residue was suspended in 230 ml. of 2N NaOH and stirred at 30°C for 3 hours; then the temperature was raised to 35°C for an additional 2-1/2 hrs. until a clear solution formed. The solution was then cooled, 85 ml. methylene chloride added, and the pH adjusted to 8.5 using 4*N* HCl with stirring. The organic layer was separated and discarded. The aqueous layer (366 ml.) was assayed by liquid chromatography to contain 37.2 mg/ml; 87% Z isomer. Another 85 ml. portion of CH_2Cl_2 was then added and pH adjusted to 4.5 with stirring. The organic layer was separated and the aqueous layer re-extracted with 50 ml. of CH_2Cl_2, with the pH again adjusted to 4.5. Combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to a gum. This residue was dissolved in 150 ml. isopropanol and 15 ml. water and the pH adjusted to 8.2 with 2 *N* NaOH. The resulting solution was concentrated to an oily residue which was flushed with isopropanol until it turned to a crystalline solid, indicating that most water had been removed. It was crystallized from 120 ml. of isopropanol, (cooled in ice for 1 hour) filtered, and washed with 50 ml. cold isopropanol followed by copious amounts of acetone. It was dried at 60°C/0.1 mm/2 hours to yield 10.74 g (63.2%) crystalline material, having essentially a single peak in liquid chromatography, m.p. 241—243°C.

The starting material, (+)-2,2-dimethylcyclopropanecarboxamide is most conveniently prepared by resolution of the *D,L* acid, followed by reaction with oxalyl chloride and then ammonia to give the resolved amide.

One way of making the starting material is as follows: 23.1 g. of *D,L*-2,2-dimethylcyclopropanecarboxylic acid was suspended in 33 ml H_2O and the pH adjusted to 8.0, using 50% NaOH, about 10 ml. To this was added a solution of 38.4 g. quinine in a mixture of 60 ml. methanol and 30 ml. H_2O to which had been added about 8 ml. of concentrated HCl in another 30 ml. H_2O to give a pH of 7.1. (This was actually a solution of quinine hydrochloride.)

These solutions were added all at once, with stirring. The gummy crystalline material which formed was heated to give two clear layers and again stirred vigorously while cooling to give a crystalline product. This product was permitted to stand over two days at room temperature. It was then filtered, washed with 2 ×10 ml. water, and 2 × 10 ml. 50% methanol, and air dried with suction. The yield of crude quinine salt was 44.8 g (48.7% yield) monohydrate, m.p 113—116°C, having a $[\alpha]_D^{20}$ of −94.3°, C = 1.0; $CHCl_3$. This material was recrystallized from acetone to yield 24.35 g, m.p. 127—130°C. This purified quinine salt was converted to the acid by reaction with aqueous base and chloroform, followed by acid, to yield (96%) 3.9 g $[\alpha]_D^{20}$ of +146.0°.

This acid was converted to the amide as follows: A charge of 30.5 g (+)acid was added over 5—10 minutes through a dropping funnel to chilled (10°C) oxalyl chloride, 54 ml., containing 1 drop dimethyl-formamide. This was stirred overnight at ambient temperature. A clear solution was observed, which was added to 100 ml. methylene chloride to dilute. Excess oxalyl chloride was removed by concentrating and the mixture flushed twice with methylene chloride.

The resultant solution was diluted with an equal volume of methylene chloride, and added continuously through a dropping funnel to about 100 ml. anhydrous liquid ammonia which was diluted with 100 ml. methylene chloride. A dry ice-acetone cooling bath was used during the addition. When all was added, the cooling bath was removed and the mixture stirred at room temperature for about 1/2 hour. The mixture was filtered, to remove precipitated ammonium chloride, and concentrated to dryness. The crude weight was 26.6 g. (88%). It was redissolved in excess hot ethyl acetate and filtered through a pre-heated sintered glass funnel to separate from trace NH_4Cl. Excess ethyl acetate was atmospherically distilled off. When half the volume remained, 130 ml. of heptane were added, and ethyl acetate was continued to be distilled off, until the boiling point started to rise (to near 80°C; much of product had already crystallized out). Heat was removed, and the mixture let cool gradually to about 30°C, then cooled with an ice bath to 0—5°C for about 1/2 hour. The product was recovered as nice silvery-white crystalline flakes, washed with 3 × ethyl acetate/hexane mixture, 1/1.5 and air dried to constant weight. It weighed 23.3 g (77.1% yield overall, 87.6% recovery from crude), m.p. = 135—138°C (varies with rate of heating). Angle of rotation was determined by dissolving 0.0543 g in 10 ml. chloroform, $[\alpha]_D^{20}$ = +100.9°.

Example 15

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

Step A: 2,2-Dichlorocyclopropanecarboxamide

A 7.1 g. sample of 2,2-dichlorocyclopropanecarbonyl chloride (U.S. Patent 3,301,896, issued January 31, 1967) was added dropwise to 75 ml of concentrated ammonium hydroxide with vigorous stirring. The temperature of the reaction mixture was maintained below 10°C with an ice bath. The mixture was stirred in the ice bath for 30 min., then at room temperature for 1 hr. The aqueous ammonia was evaporated under reduced pressure (bath at 50°C). The solid residue was extracted with hot ethyl acetate (3 x 30 ml.). The extracts were boiled down to 40 ml. and 20 ml. of hexane was added. After cooling in ice, the solid was filtered, washed with ethyl acetate-hexane (1:1) and dried to give 2.7 g of 2,2-dichlorocyclopropanecarboxamide, m.p. 144—146°. The NMR spectrum was in accord with the desired structure.

Anal. ($C_{12}H_5Cl_2NO$)	Calcd.	Found
C	31.20	31.26
H	3.27	3.31
N	9.10	9.11
Cl	46.04	45.79

Another 1.3 g of amide, m.p. 143—145° could be recovered from the mother liquor.

Step B; Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-butenoic acid

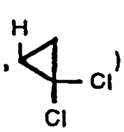
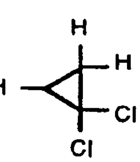
A mixture of 1.53 g (15 mmoles) of 2-ketobutyric acid, 1.54 g (10 mmoles) of 2,2-dichlorocyclopropanecarboxamide and 10 ml of toluene was heated under reflux for 12 hrs. with removal of H_2O by a modified Dean-Stark trap containing molecular sieves (4A). An additional 0.7 g. of 2-ketobutyric acid was added and the reaction mixture was heated under reflux for an additional 12 hrs. The mixture was cooled, diluted with 20 ml. of toluene and extracted with saturated sodium bicarbonate (3 x 10 ml.). The extracts were combined, washed with ether and acidified to pH 3 (pH meter) with concentrated hydrochloric acid. A gum precipitated which soon solidified. It was filtered, washed with water, dried and recrystallized from nitromethane to give 423 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-butenoic acid, m.p. 188—189.5°C. The NMR spectrum was in accord with the desired structure.

Anal. ($C_8H_9Cl_2NO_3$)	Calcd.	Found
C	40.36	40.48
H	3.81	3.80
N	5.88	5.91
Cl	29.78	29.53

Example 16

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-2-octenoic acid

A mixture of 1.19 g (7.5 mmoles) of 2-keto-octanoic acid, 0.77 g (5.0 mmoles) of 2,2-dichlorocyclopropanecarboxamide, and 5 ml toluene were reacted using the same procedure as in the previous example. The crude product (537 mg) was purified by conversion to the methyl ester (BF_3/CH_3OH), preparative TLC (silica gel G, 4:1 hexane-EtOAc) and saponification of the pure Z-methyl ester (0.3M $LiOH/CH_3OH$) to give 88 mg of Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-octenoic acid as a partially crystalline gum. NMR Spectrum (DMSO-d_6: 9.68 δ (s, 1H, NH), 6.50 δ (t, 1H, $=^{H}$),

2.83 δ (t, 1H, ), 1.97 δ (d, 2H ),

0.87 δ (t, 3H, CH_3).

Example 17

Z-8-Bromo-2-(2,2-Dimethylcyclopropanecarboxamido)-2-octenoic acid

To a suspension of 14.4 g (0.3 mole) of 50% NaH dispersion in 360 ml of toluene cooled in an ice bath and in a N_2 atmosphere was added over 45 min. a solution of 146 g (0.6 moles) of 1,6-dibromohexane and 57.6 g (0.3 mole) of ethyl 1,3-dithiane-2-carboxylate in 120 ml of DMF. The cooling bath was removed and the mixture stirred at room temperature for 2 hrs. The reaction mixture was washed with water (3 x 210 ml), dried over $MgSO_4$ and evaporated under reduced pressure to give 179.5 g of a yellow oil containing the desired anhydrated dithiane, 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

To a suspension of 426 g (2.4 moles) of N-bromosuccinimide in 800 ml of acetonitrile and 200 ml of H_2O was added over 45 min. a solution of the crude dithiane in 100 ml of acetonitrile. The temperature of the reaction mixture was maintained below 25°C with an ice bath. After stirring at 20°C for 10 min. the dark red reaction mixture was poured into 2 l. of hexane-CH_2Cl_2 (1:1). The solution was shaken with saturated $NaHSO_3$ (2 x 400 ml) and water (1 x 500 ml). Then 400 ml of saturated Na_2CO_3 solution was added in small portions (vigorous CO_2 evolution). After the foaming subsided the funnel was shaken and the aqueous phase separated. The organic layer was extracted with saturated Na_2CO_3 solution (400 ml) and water (500 ml) and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave 133.8 g of crude bromo keto-ester containing 1,6-dibromohexane and mineral oil. This crude material was used in the next reaction without purification.

A mixture of 133.8 g of crude bromo ketoester, 133 ml of 50% hydrobromic acid and 267 ml of acetic acid was heated at 90°C (internal temperature) for 75 min. The dark solution was evaporated under reduced pressure until most of the acetic acid was removed. The residue was dissolved in 500 ml of ether, washed with water (2 x 100 ml) and extracted with saturated $NaHCO_3$ (3 x 200 ml). The combined $NaHCO_3$ extracts were extracted with ether (2 x 100 ml) and acidified with concentrated HCl. The precipitated oil was extracted with ether (3 x 200 ml). The ether extracts were washed with water (1 x 100 ml) and saturated brine (1 x 100 ml) and dried over $MgSO_4$. Removal of the ether under reduced pressure gave 46.2 g of pure bromoketo acid. Homogeneous by TlC (silica gel, 4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired product.

A mixture of 46.1 g (0.194 moles) of the bromoketo acid, 17.6 g (0.156 mole) of 2,2-dimethylcyclopropanecarboxamide and 450 ml of toluene was heated under reflux for 13 hrs., with collection of water in a small Dean-Stark trap. After cooling, the clear reaction mixture was extracted with saturated $NaHCO_3$ solution (4 x 100 ml). The combined extracts were washed with ether (2 x 100 ml) and then the pH was adjusted to 3.5 (pH meter) by addition of concentrated HCl. An oil precipitated which soon crystallized. The solid was filtered, washed well with water and dried. Recrystallization from acetonitrile gave 22.5 g of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid, m.p. 151—153°C. Homogenous by TLC (4:1 toluene-acetic acid). The NMR spectrum was consistent with the desired structure.

Anal. ($C_{14}H_{22}BrNO_3$)	Calcd.	Found
C	50.61	50.66
H	6.67	6.96
N	4.22	4.45
Br	24.05	23.95

The following ω-bromo compounds were prepared using the same procedure:

Z-6-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)hexenoic acid;
Z-7-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)heptenoic acid;
Z-9-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-nonenoic acid;
Z-10-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)decenoic acid;
Z-8-Bromo-2-(2,2-dimethylcyclopropanecarboxamido)octenoic acid.

Example 18

Z-8-Dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid

A solution of 664 mg (2 mmoles) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 10 ml of 40% aqueous dimethylamine was allowed to stand at room temperature for 4 hrs. The solution was poured onto a 3.5 x 20 cm column of Dowex 50W-x8 (100—200 mesh, H^+) ion exchange resin and column eluted with water until the effluent was no longer acidic (~200 ml). The column was then eluted wtih 300 ml of 2N ammonium hydroxide. The effluent was evaporated under reduced pressure to give 600 mg of a colorless glass. This material was dissolved in 3 ml of ethanol, filtered, and added dropwise to 200 ml of rapidly stirred acetone. A gummy solid precipitated which crystallized upon stirring for two days. The solid was filtered, washed with acetone, and dried to give

445 mg of Z-8-dimethylamino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid as a colorless, hygroscopic crystals, m.p. 101—112°C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{16}H_{28}N_2O_3.H_2O$)	Calcd.	Found
C	61.62	61.03
H	9.62	9.28
N	8.91	8.67

The following 8-amino derivatives were prepared using essentially the same procedure, "DCC" means 2-(2,2-dimethylcyclopropanecarboxamido).

Z-10-Dimethylamino-DCC-2-decenoic acid;
Z-8-Amino-DCC-2-octenoic acid;
Z-8-Dimethylamino-DCC-2-octenoic acid;
Z-7-Dimethylamino-DCC-2-heptenoic acid;
Z-DCC-7-(N-methylpiperazinyl)-2-heptenoic acid;
Z-DCC-8-pyrrolidino-2-octenoic acid;
Z-DCC-8-(N-methylpiperazinyl)-2-octenoic acid;
Z-8-Allylamino-DCC-2-octenoic acid;
Z-DCC-8-piperidino-2-octenoic acid;
Z-DCC-8-propargylamino-2-octenoic acid;
Z-8-N-[1-Deoxy-(1-methylamino)-D-glucityl]-DCC-2-octenoic acid;
Z-8-(1-Adamantylamino)-DCC-2-octenoic acid;
Z-8-Diallylamino-DCC-2-octenoic acid;
Z-8-DCC-8-(2-hydroxyethylmethylamino)-2-octenoic acid;
Z-8-[(Carboxylmethyl)methylamino]-2-(2,2-DCC)-2-octenoic acid;
Z-2-(2,2-DCC)-8-diethylamino-2-octenoic acid;
Z-2-(2,2-DCC)-8-[tris-(hydroxymethyl)methylamino)-2-octenoic acid
Z-2-(2,2-DCC)-10-(N-methylpiperazinyl)-2-decenoic acid;
Z-2-(2,2-DCC)-8-[1-(phosphono)ethylamino]-2-octenoic acid;

Example 19

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid

A stream of CH_3SH gas was bubbled through a solution of 162 mg (3 mmoles) of sodium methoxide in 5 ml of methane for 10 min. with cooling in an ice bath. The solution was allowed to warm to room temperature and 332 mg (1 mmole) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was added. The solution was heated under reflux for 30 min. in a N_2 atmosphere. Most of the methanol was evaporated under reduced pressure, the residue was dissolved in 10 ml of water and acidified with 2.5 *N* HCl. The precipitated oil was extracted with ether (3 ×). The ether extracts were washed with water, saturated in brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 178 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methylthio-2-octenoic acid, m.p. 82—84°C. Homogeneous by TLC (toluene-acetic acid, 4:1). The NMR spectrum was in accord with the desired structure.

Anal. ($C_{15}H_{25}NO_3S$)	Calcd.	Found
C	60.18	60.36
H	8.42	8.68
N	4.68	4.59
S	10.69	10.87

The following compounds were prepared by similar methods, "DCC" means 2-(2,2-dimethylcyclopropanecarboxamido).

Z-DCC-8-ethoxythiocarbonylthio-2-octenoic acid;
Z-DCC-8-(1-methyl-5-tetrazolylthio)-2-octenoic acid;
Z-DCC-7-[[(methoxycarbonyl)methyl]thio]-2-heptenoic acid;
Z-8-Acetylthio-DCC-2-octenoic acid;
Z-7-[(2-Amino-2-oxoethyl)thio]-DCC-2-heptenoic acid;

6-(L-2-Amino-2-carboxyethylthio)-2-(2,2-DCC-2-hexenoic acid;
Z-8-(Carbomethoxymethylthio)-2-(2,2-DCC)-2-octenoic acid;
Z-6-(Carbomethoxymethylthio)-2-(2,2-DCC)-2-hexenoic acid;
Z-2-(2,2-DCC)-6-(phosphonomethylthio)-2-hexenoic acid.

Example 20

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt

A solution of 996 mg (3 mmoles) of Z-8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid in 15 ml of 25% aqueous trimethylamine was allowed to stand at room temperature for 3 hrs. The reaction mixture was poured onto a 2 ×25 cm column of IRA-410 (50—100 mesh, OH^-) ion exchange resin and eluted with water until the effluent was no longer basic. The effluent was evaporated under reduced pressure to give 800 mg of a colorless glass. This material was dissolved in 20 ml. of ethanol, filtered and diluted with 600 ml of acetone. After standing at room temperature overnight the crystalline solid which deposited was filtered, washed with acetone and dried to give 720 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt as hygroscopic crystals, m.p. 220—222°C. Homogeneous by TLC (silica gel, in BuOH, HOAc, H_2O, 4:1:1). NMR spectrum was consistent with desired structure.

Anal. ($C_{17}H_{30}N_2O_3$)	Calcd.	Found
C	65.77	65.78
H	9.74	9.98
N	9.02	8.92

Other quaternary derivatives were prepared using essentially the same procedure; these are

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-trimethyl-ammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-pyridinium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(2-hydroxyethyldimethylammonium hydroxide)-2-octenoic acid inner salt;

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-trimethylammonium hydroxide-2-decenoic acid inner salt;

Z - 8 - (Benzyldimethylammonium hydroxide) - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2-octenoic acid inner salt;

Z - 10 - (Benzyldimethylammonium hydroxide) - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2-decenoic acid inner salt;

Z - 2 - (2,2 - Dimethylcyclopropanecarboxamido) - 9 - trimethylammonium hydroxide - 2 - nonenoic acid inner salt;

Z - 8 - (2 - Dimethylaminoethyldimethylammonium hydroxide) - 2 - (2,2 - dimethylcyclopropanecarboxamido) - 2 - octenoic acid inner salt;

Z-2-(2,2-Dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Example 21

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid

A 350 mg sample of Z-8-amino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid was dissolved in 10 ml of water and the pH adjusted to 8.5 with 2.5N NaOH. A total of 947 mg of benzyl formimidate hydrochloride was added at room temperature in small portions over 20 min. while the pH was maintained between 8—9 by addition of 2.5N NaOH. After stirring at room temperature for 30 min., the cloudy reaction mixture was extracted with ether (3 ×) and applied to a 2 × 2.5 cm column of a G50W-X4 (Na^+, 200—400 mesh) resin. After elution with water, the fractions containing the product were pooled and evaporated under reduced pressure. This material was dissolved in water and applied to a 2 × 25 cm column of a G1X8 (HCO_3^-, 200—400 mesh) resin. After elution with water, the fractions containing pure product were pooled and evaporated under reduced pressure. The residue was dissolved in a few ml of warm ethanol, filtered, and added dropwise to 200 ml of ether with rapid stirring. Filtration and washing with ether gave 243 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-formamidino-2-octenoic acid as an amorphous solid. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). The NMR spectrum was in accord with the desired structure.

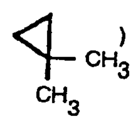
Anal. ($C_{15}H_{25}N_3O_3 \cdot 1/3H_2O$)		Calcd.	Found
	C	59.96	60.04
	H	8.59	8.64
	N	13.92	13.57

The following amidino compounds were prepared using the similar procedures:
Z-8-Acetamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;
Z-8-Benzylamidino-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-10-formamidino-2-decenoic acid;
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-(2-imidazoyl-2-yl-amino)-2-octenoic acid.

Example 22

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid

To a solution of 2 mmoles of guanidine (prepared from 432 mg of guanidine sulfate and 630 mg of barium hydroxide octahydrate) in 7 ml of water was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)octenoic acid, and the solution was heated at 70°C in a nitrogen atmosphere for 1 hr. The reaction mixture was applied to a 2 × 25 cm column of Dowex 50W-X8 (H^+, 100—200 mesh). After elution with water the fractions containing the product were pooled and evaporated under reduced pressure. The residue was dissolved in several ml of warm ethanol and added dropwise to 100 ml of ether with rapid stirring. Filtration and washing with ether gave 107 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid as an amorphous electrostatic powder. Homogeneous by TLC (n-BuOH, HOAc, H_2O; 4:1:1). NMR (D_2O, NaOD): 6.48 δ (t, 1H, $=^{\prime H}$); 3.10 δ (m, 2H, $\overset{\text{H}}{\text{C}}$HN—), 2.10 δ (m, 2H, $=^{\prime CH_2}$), 1.17 δ

(s, 3H, ).

The following guanidino compound was prepared using the same procedure:
Z - 2 - (2,2 -dimethylcyclopropanecarboxamido) - 8 - (N,N - dimethylguanidino) - 2 - octenoic acid.

Example 23

Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-methyl-2-octenoic acid

To a solution of 2.43 mmoles of sodium methoxide in 5 ml of methanol was added 332 mg (1 mmole) of 8-bromo-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid. The solution was heated under reflux in a nitrogen atmosphere for 1 hr. The reaction mixture was evaporated under reduced pressure, the residue dissolved in water and acidified with 2.5 *N* hydrochloric acid. The oil which precipitated was extracted with ether (3 x). The ether extracts were washed with water, and saturated brine and dried over $MgSO_4$. Removal of the ether under reduced pressure gave a colorless oil that crystallized upon standing. It was recrystallized from ether-hexane to give 140 mg of Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-methoxy-2-octenoic acid, m.p. 71—72°C. Homogeneous by TLC (toluene-HOAc, 4:1). The NMR spectrum was in accord with the desired structure.

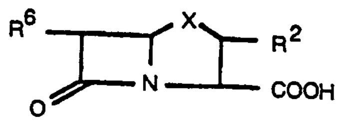
Anal. ($C_{15}H_{25}NO_4$)		Calcd.	Found
	C	63.58	63.54
	H	8.89	9.12
	N	4.94	5.16

Using similar procedures, the following compounds were prepared:
Z-8-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid;
Z-7-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)-2-heptenoic acid;
Z-9-Cyano-2-(2,2-dimethylcyclopropanecarboxamido)nonenoic acid;
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-7-sulfo-2-heptenoic acid sodium salt;
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-sulfo-2-octenoic acid sodium salt;
Z-2-(2,2-Dimethylcyclopropanecarboxamido)-8-hydroxy-2-octenoic acid;
Z-8-Acetoxy-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

Claims for the Contracting States: BE CH DE FR GB IT LU NL SE

1. Antibacterial composition characterized in that it contains a combination of a thienamycin type compound with is a 6-substituted and optionally 2-substituted pen-2-em-3-carboxylic acid, a 1-carbadethia-pen-2-em-3-carboxylic acid or 1-azabicyclo[3.2.0]hept-2-ene-7-one-2-carboxylic acid and a dipeptidase (E.C.3.4.13.11) inhibitor, the weight ratio of the first named material to the dipeptidase inhibitor being within the range of 1:3 to 30:1.

2. The composition of Claim 1 in which the first named material is a compound of the following formula



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wherein X can be CH_2 or S; R^2 can be hydrogen; —S—$CH_2CH_2NHR^3$, wherein R^3 is hydrogen, acetyl, formimidoyl, acetimidoyl; —S(O)—CH=$CHNHCOCH_3$ and —S—CH=$CHNHCOCH_3$; and R^6 is

$$-\underset{\displaystyle R^7}{\underset{|}{C}}HCH_3$$

wherein R^7 is hydrogen, hydroxy or hydroxy-sulfonyloxy, or R^6 is H, including all possible stereo-isomeric forms.

3. The composition of Claim 1 or 2 in which the combination is mixed with a pharmaceutical carrier.

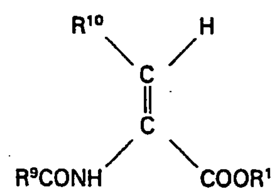
4. The composition of Claim 3 in which the carrier is adapted for injection.

5. The composition of Claims 1 or 2 in which the thienamycin-type compound is thienamycin.

6. The composition of Claim 1 or 2 in which the thienamycin-type compound is N-formimidoylthienamycin.

7. The composition of Claim 1 or 2 in which the thienamycin-type compound is N-acetimidoyl-thienamycin.

8. The composition of Claim 1 or 2 in which the dipeptidase inhibitor is a compound of the following formula:



wherein R^9 and R^{10} are hydrocarbon radicals in the range respectively of 3—10 and 1—15 carbon atoms; in either one of these R^9 or R^{10} hydrocarbon chains 1—6 hydrogens may be replaced by halogens or a non-terminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^{10} can also be replaced by a hydroxyl or thiol group, which may be acylated or carbamoylated; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted amino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as terminal amino acid grouping; and R^1 is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl, or a pharmaceutically acceptable cation.

9. The composition of claim 8 in which R^9 is a straight, branched or cycloalkyl group of 3—10 carbon atoms including alkylcycloalkyl and dialkylcycloalkyl, providing the carbon adjacent to the carbonyl cannot be tertiary.

10. The composition of Claim 8 in which R^9 can be R^4, wherein R^4 is branched or cyclic hydrocarbon of 3—10 carbon atoms;

—R^5R^{11}, wherein R^5 is cycloalkyl of 3—6 carbon atoms and R^{11} is either 1 or 2 alkyl substituents which may be joined to form another ring on the cycloalkyl group or R^{11} is 1 or 2 chloro substituents;

—$R^{12}R^8$, wherein R^{12} is alkylene of 1—3 carbon atoms and R^8 is cycloalkyl of 3—6 carbon atoms.

11. The composition of Claim 8 in which R^9 is 2,2-dimethylcyclopropyl.

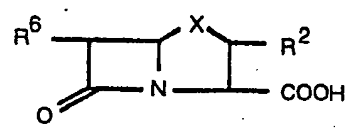
12. The composition of Claim 8 in which R^9 is 2,2-dichlorocyclopropyl.

13. The composition of Claim 8 in which the dipeptidase inhibitor is Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

Claims for the Contracting State: AT

1. Process for preparing an antibacterial composition characterized in mixing a thienamycin type compound which is a 6-substituted and optionally a 2-substituted pen-2-em-3-carboxylic acid, a 1-carbadethia-pen-2-em-3-carboxylic acid or 1-azabicyclo[3.2.0]hept-2-ene-7-one-2-carboxylic acid and a dipeptidase (E.C.3.4.13.11) inhibitor, the weight ratio of the first named material to the dipeptidase inhibitor being within the range of 1:3 to 30:1.

2. Process according to claim 1 in which the first named material is a compound of the following formula



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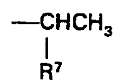
wherein X can be CH_2 or S; R^2 can be hydrogen; —S—$CH_2CH_2NHR^3$, wherein R^3 is hydrogen, acetyl, formimidoyl, acetimidoyl; —S(O)—CH=$CHNHCOCH_3$ and —S—CH=$CHNHCOCH_3$; and R^6 is



wherein R^7 is hydrogen, hydroxy or hydroxy-sulfonyloxy, or R^6 is H, including all possible stereoisomeric forms.

3. Process according to Claim 1 or 2 in which the components are mixed with a pharmaceutical carrier.

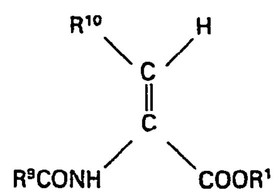
4. Process according to Claim 3 in which the carrier is adapted for injection.

5. Process according to Claim 1 or 2 in which the thienamycin-type compound is thienamycin.

6. Process according to Claim 1 or 2 in which the thienamycin-type compound is N-formimidoyl-thienamycin.

7. Process according to Claims 1 or 2 in which the thienamycin-type compound is N-acetimidoyl-thienamycin.

8. Process according to Claim 1 or 2 in which the dipeptidase inhibitor is a compound of the following formula:



wherein R^9 and R^{10} are hydrocarbon radicals in the range respectively of 3—10 and 1—15 carbon atoms; in either one of these R^9 or R^{10} hydrocarbon chains 1—6 hydrogens may be replaced by halogens or a non-terminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^{10} can also be replaced by a hydroxyl or thiol group, which may be acylated or carbamoylated; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted alkylamino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as a terminal amino acid grouping; and R^1 is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl, or a pharmaceutically acceptable cation.

9. Process according to Claim 8 in which R^9 is a straight, branched or cycloalkyl group of 3—10 carbon atoms including alkylcycloalkyl and dialkylcycloalkyl, providing the carbon adjacent to the carbonyl cannot be tertiary.

10. Process according to Claim 8 in which R^9 in the dipeptidase inhibitor can be R^4, wherein R^4 is branched or cyclic hydrocarbon of 3—10 carbon atoms;

—R^5R^{11}, wherein R^5 is cycloalkyl of 3—6 carbon atoms and R^{11} is either 1 or 2 alkyl substituents which may be joined to form another ring on the cycloalkyl group or R^{11} is 1 or 2 chloro substituents;

—$R^{12}R^8$, wherein R^{12} is alkylene of 1—3 carbon atoms and R^8 is cycloalkyl of 3—6 carbon atoms.

11. Process according to Claim 8 in which R^9 in the dipeptidase inhibitor is 2,2-dimethylcyclopropyl.

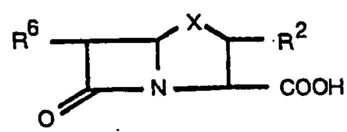
12. Process according to Claim 8 in which R^9 in the dipeptidase inhibitor is 2,2-dichlorocyclopropyl.

13. Process according to Claim 8 in which the dipeptidase inhibitor is Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-octenoic acid.

Revendications pour les Etats contractants: BE CH DE FR GB IT LU NL SE

1. Composition antibactérienne caractérisée en ce qu'elle contient une combinaison d'un composé du type thiénamycine qui est un acide pen-2-em-3-carboxylique 6-substitué et éventuellement 2-substitué, un acide 1-carbadéthia-pen-2-em-3-carboxylique ou 1-azabicyclo[3.2.0]-hept-2-ène-7-one-2-carboxylique et un inhibiteur de dipeptidase (E.C.3.4.13.11), le rapport pondéral de la matière nommée en premier à l'inhibiteur de dipeptidase étant dans un intervalle de 1:3 à 30:1.

2. Composition selon la revendication 1 où la matière nommée en premier est un composé de formule



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où X peut être CH_2 ou S; R^2 peut être unhydrogène; —S—$CH_2CH_2NHR^3$, où R^3 est un hydrogène, un acétyle, un formimidoyle, un acétimidoyle; —S(O)—CH=$CHNHCOCH_3$ et —S—CH=$CHNHCOCH_3$; et R^6 est

$$\begin{array}{l}—CHCH_3\\ \quad\;|\\ \quad R^7\end{array}$$

où R^7 est un hydrogène, un hydroxy ou un hydroxy-sulfonyloxy, ou R^6 est H, y compris toutes les formes stéréoisomériques possibles.

3. Composition selon l'une des revendications 1 ou 2 où la combinaison est mélangée à un support pharmaceutique.

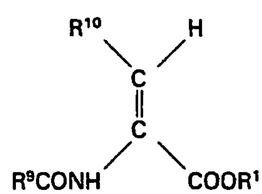
4. Composition selon la revendication 3 où le support est adapté à l'injection.

5. Composition selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la thiénamycine.

6. Composition selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la N-formimidoylthiénamycine.

7. Composition selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la N-acétimidoylthiénamycine.

8. Composition selon l'une des revendications 1 ou 2 où l'inhibiteur de dipeptidase est un composé de formule



où R^9 et R^{10} sont des radicaux hydrocarbonés dans un intervalle, respectivement, de 3—10 et 1—15 atomes de carbone; dans l'une ou l'autre de ces chaînes hydrocarbonées R^9 ou R^{10}, de 1 à 6 atomes d'hydrogène peuvent être remplacés par des halogènes, ou un méthylène non-terminal peut être remplacé par un oxygène ou un soufre, y compris les formes oxydées de ce dernier; en outre, un hydrogène terminal dans R^{10} peut également être remplacé par un groupe hydroxyle ou thiol, qui peut être acylé ou carbamoylé; ou l'hydrogène peut être remplacé par un groupe amino, qui peut être sous forme de groupe dérivé, comme dans acylamino, uréido, amidino, guanidino, ou un groupe alcoyle ou alcoylamino substitué, y compris les groupements azote quaternaire; ou encore il peut y avoir remplacement par des groupes acides comme des groupes acide carboxylique, phosphonique ou sulfonique ou leurs esters ou amides, ainsi que cyano; ou leurs combinaisons, comme un groupement acide aminé terminal; et R^1 est

un hydrogène ou un alcoyle inférieur (en C_1 à C_6) ou dialcoylaminoalcoyle, ou un cation pharmaceutiquement acceptable.

9. Composition selon la revendication 8 où R^9 est un groupe à chaîne droite, ramifiée ou cycloalcoyle en C_3 à C_{10} y compris alcoylcycloalcoyle et dialcoylcycloalcoyle, avec la précision que l'atome de carbone adjacent au carbonyle ne peut être tertiaire.

10. Composition selon la revendication 8 où R^9 peut être R^4, où R^4 est un hydrocarbure à chaîne ramifiée ou cyclique en C_3 à C_{10};

—R^5R^{11}, où R^5 est un cycloalcoyle en C_3 à C_6 et R^{11} représente 1 ou 2 substituants alcoyle qui peuvent être réunis pour former un autre noyau sur le groupe cycloalcoyle ou R^{11} représente 1 ou 2 substituants chloro;

—$R^{12}R^8$, où R^{12} est un alcoylène en C_1 à C_3 et R^8 est un cyclo-alcoyle en C_3 à C_6.

11. Composition selon la revendication 8 où R^9 est un 2,2-diméthylcyclopropyle.

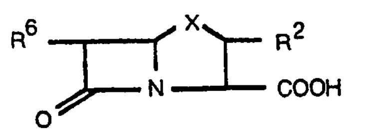
12. Composition selon la revendication 8 où R^9 est un 2,2-dichlorocyclopropyle.

13. Composition selon la revendication 8 où l'inhibiteur de dipeptidase est l'acide Z-2-(2,2-diméthylcyclopropanecarboxamido)-2-octénoïque.

Revendications pour l'Etat contractant: AT

1. Procédé de préparation d'une composition antibactérienne caractérisé en ce qu'on mélange un composé du type thiénamycine qui est un acide pen-2-em-3-carboxylique 6-substitué et éventuellement 2-substitué, un acide 1-carbadéthia-pen-2-em-3-carboxylique ou 1-azabicyclo[3.2.0]-hept-2-ène-7-one-2-carboxylique et un inhibiteur de dipeptidase (E.C.3.4.13.11), le rapport pondéral de la matière nommée en premier à l'inhibiteur de dipeptidase étant dans un intervalle de 1:3 à 30:1.

2. Procédé selon la revendication 1 où la matière nommée en premier est un composé de formule



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où X peut être CH_2 ou S; R^2 peut être un hydrogène; —S—$CH_2CH_2NHR^3$, où R^3 est un hydrogène, un acétyle, un formimidoyle, un acétimidoyle; —S(O)—CH=CHNHCOCH$_3$ et —S—CH=CHNHCOCH$_3$; et R^6 est

$$-\underset{\substack{|\\R^7}}{CH}CH_3$$

où R^7 est un hydrogène, un hydroxy ou un hydroxy-sulfonyloxy, ou R^6 est H, y compris toutes les formes stéréoisomériques possibles.

3. Procédé selon l'une des revendications 1 ou 2 où l'on mélange les composants avec un support pharmaceutique.

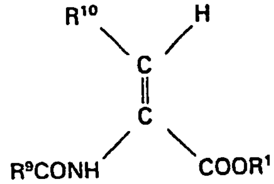
4. Procédé selon la revendication 3 où le support est adapté à l'injection.

5. Procédé selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la thiénamycine.

6. Procédé selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la N-formimidoylthiénamycine.

7. Procédé selon l'une des revendications 1 ou 2 où le composé du type thiénamycine est la N-acétimidoylthiénamycine.

8. Procédé selon l'une des revendications 1 ou 2 où l'inhibiteur de dipeptidase est un composé de formule



où R^9 et R^{10} sont des radicaux hydrocarbonés dans un intervalle, respectivement, de 3—10 et 1—15 atomes de carbone; dans l'une ou l'autre de ces chaînes hydrocarbonées R^9 ou R^{10}, de 1 à 6 atomes d'hydrogène peuvent être remplacés par des halogènes, ou un méthylène non-terminal peut être remplacé par un oxygène ou un soufre, y compris les formes oxydées de ce dernier; en outre, un hydrogène

terminal dans R^{10} peut également être remplacé par un groupe hydroxyle ou thiol, qui peut être acylé ou carbamoylé; ou l'hydrogène peut être remplacé par un groupe amino, qui peut être sous forme de groupe dérivé, comme dans acylamino, uréido, amidino, guanidino, ou un groupe alcoyle ou alcoylamino substitué, y compris les groupements azote quaternaire; ou encore il peut y avoir remplacement par des groupes acides comme des groupes acide carboxylique, phosphonique ou sulfonique ou leurs esters ou amides, ainsi que cyano; ou leurs combinaisons, comme un groupement acide aminé terminal; et R^1 est un hydrogène ou un alcoyle inférieur (en C_1 à C_6) ou dialcoylaminoalcoyle, ou un cation pharmaceutiquement acceptable.

9. Procédé selon la revendication 8 où R^9 est un groupe à chaîne droite, ramifiée ou cycloalcoyle en C_3 à C_{10} y compris alcoylcycloalcoyle et dialcoylcycloalcoyle, avec la précision que l'atome de carbone adjacent au carbonyle ne peut être tertiaire.

10. Procédé selon la revendication 8 où R^9 dans l'inhibiteur de dipeptidase peut être R^4, où R^4 est un hydrocarbure à chaîne ramifiée ou cyclique en C_3 à C_{10};

—R^5R^{11}, où R^5 est un cycloalcoyle en C_3 à C_6 et R^{11} représente 1 ou 2 substituants alcoyle qui peuvent être réunis pour former un autre noyau sur le groupe cycloalcoyle ou R^{11} représente 1 ou 2 substituants chloro;

—$R^{12}R^8$, où R^{12} est un alcoylène en C_1 à C_3 et R^8 est un cyclo-alcoyle en C_3 à C_6.

11. Procédé selon la revendication 8 où R^9 dans l'inhibiteur de dipeptidase est le 2,2-diméthylcyclopropyle.

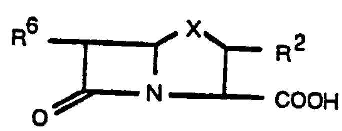
12. Procédé selon la revendication 8 où R^9 dans l'inhibiteur de dipeptidase est le 2,2-dichlorocyclopropyle.

13. Procédé selon la revendication 8 où l'inhibiteur de dipeptidase est l'acide Z-2-(2,2-diméthylcyclopropanecarboxamido)-2-octénoïque.

Patentansprüche für die Vertragsstaaten: BE CH DE FR GB IT LU NL SE

1. Antibakterielle Zusammensetzung, dadurch gekennzeichnet, daß sie eine Kombination aus einer Verbindung vom Thienamycin-Typ, welche Verbindung eine 6-substituierte und gegebenenfalls 2-substituierte Pen-2-em-3-carbonsäure, eine 1-Carbadethia-pen-2-em-3-carbonsäure oder eine 1-Azabicyclo[3.2.0]hept-2-en-7-on-2-carbonsäure ist, und einem Dipeptidase (E.C.3.4.13.11)-Inhibitor enthält, wobei das Gewichtsverhältnis des erstgenannten Materials zu dem Dipeptidaseinhibitor im Bereich von 1:3 bis 30:1 liegt.

2. Die Zusammensetzung nach Anspruch 1, in der das erstgenannte Material eine Verbindung der nachstehenden Formel



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ist, worin X CH_2 S sein kann; R^2 Wasserstoff; —S—$CH_2CH_2NHR^3$, worin R^3 Wasserstoff, Acetyl, Formimidoyl, Acetimidoyl ist; —S(O)—CH=CHNHCOCH$_3$ und —S—CH=CHNHCOCH$_3$ sein kann; und R^6

$$\begin{array}{c}—CHCH_3\\ |\\ R^7\end{array}$$

ist, worin R^7 Wasserstoff, Hydroxy oder Hydroxysulfonyloxy ist, oder R^6 Wasserstoff ist, einschließlich aller möglichen stereoisomeren Formen.

3. Die Zusammensetzung nach Anspruch 1 oder 2, in der die Kombination mit einem pharmazeutischen Träger vermischt ist.

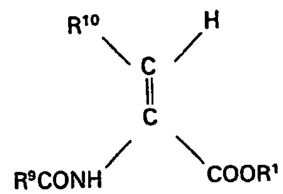
4. Die Zusammensetzung nach Anspruch 3, in der der Träger zur Injektion angepaßt ist.

5. Die Zusammensetzung nach Anspruch 1 oder 2, in der die Thienamycin-Typ-Verbindung Thienamycin ist.

6. Die Zusammensetzung nach Anspruch 1 oder 2, in der die Thienamycin-Typ-Verbindung N-Formimidoylthienamycin ist.

7. Die Zusammensetzung nach Anspruch 1 oder 2, in der die Thienamycin-Typ-Verbindung N-Acetimidoylthienamycin ist.

8. Die Zusammensetzung nach Anspruch 1 oder 2, in der der Dipeptidaseinhibitor eine Verbindung der folgenden Formel ist.



worin R^9 und R^{10} Kohlenwasserstoffreste im Bereich von 3—10 bzw. 1—15 Kohlenstoffatomen sind; wobei in jeder dieser R^9- oder R^{10}-Kohlenwasserstoffketten 1—6 Wasserstoffe ersetzt sein können durch Halogene oder ein nicht endständiges Methylen ersetzt sein kann durch Sauerstoff oder Schwefel, einschließlich der oxydierten Formen des letzteren; ferner ein endständiger Wasserstoff in R^{10} auch ersetzt sein kann durch eine Hydroxyl- oder Thiolgruppe, die acyliert oder carbamoyliert sein kann; oder der Wasserstoff ersetzt sein kann durch eine Aminogruppe, die in Derivatform vorliegen kann, wie in einer Acylamino-, Ureido-, Amidino-, Guanidino- oder Alkyl- oder substituierten Alkylamino-gruppe, einschließlich quaternärer Stickstoffgruppen; oder alternativ ein Ersatz durch saure Gruppen vorliegen kann, wie Carbonsäure-, Phosphonsäure- oder Sulfonsäuregruppen oder Ester oder Amide davon, sowie Cyano; oder Kombinationen davon, wie eine endständige Aminosäuregruppe; und R^1 Wasserstoff oder Niedrigalkyl(C_{1-6}) oder Dialkylaminoalkyl oder ein pharmazeutisch brauchbares Kation ist.

9. Die Zusammensetzung nach Anspruch 8, in der R^9 eine gerade, verzweigte oder Cyclo-alkylgruppe mit 3 bis 10 Kohlenstoffatomen einschließlich Alkylcycloalkyl und Dialkylcycloalkyl ist, vorausgesetzt, daß der zur Carbonylgruppe benachbarte Kohlenstoff nicht tertiär sein kann.

10. Die Zusammensetzung nach Anspruch 8, in der R^9 sein kann R^4, worin R^4 verzweigter oder cyclischer Kohlenwasserstoff mit 3—10 Kohlenstoffatomen ist;

—R^5R^{11}, worin R^5 Cycloalkyl mit 3—6 Kohlenstoffatomen ist und R^{11} entweder 1 oder 2 Alkylsubstituenten ist, die verbunden sein können unter Bildung eines weiteren Ringes an der Cycloalkylgruppe oder R^{11} 1 oder 2 Chlorsubstituenten bedeutet;

—$R^{12}R^8$, worin R^{12} Alkylen mit 1 bis 3 Kohlenstoffatomen ist und R^8 Cycloalkyl mit 3 bis 6 Kohlenstoffatomen ist.

11. Die Zusammensetzung nach Anspruch 8, in der R^9 2,2-Dimethylcyclopropyl ist.

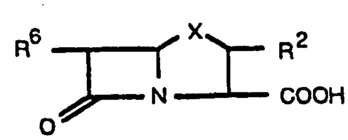
12. Die Zusammensetzung nach Anspruch 8, in der R^9 2,2-Dichlorcyclopropyl ist.

13. Die Zusammensetzung nach Anspruch 8, in der der Dipeptidaseinhibitor Z-2-(2,2-Dimethylcyclopropancarboxamido)-2-octensäure ist.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung einer antibakteriellen Zusammensetzung, gekennzeichnet durch Vergemischen einer Verbindung vom Thienamycin-Typ, welche Verbindung eine 6-substituierte und gegebenenfalls 2-substituierte Pen-2-em-3-carbonsäure, eine 1-Carbadethia-pen-2-em-3-carbonsäure oder eine 1-Azabicyclo[3.2.0]hept-2-en-7-on-2-carbonsäure ist, und eines Dipeptidase (E.C.3.4.13.11)-Inhibitors, wobei das Gewichtsverhältnis des erstgenannten Materials zu dem Dipeptidaseinhibitor im Bereich von 1:3 bis 30:1 liegt.

2. Verfahren nach Anspruch 1, in dem das erstgenannte Material eine Verbindung der nachstehenden Formel



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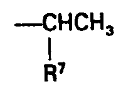
ist, worin X CH_2 oder S sein kann; R^2 Wasserstoff; —S—$CH_2CH_2NHR^3$, worin R^3 Wasserstoff, Acetyl, Formimidoyl, Acetimidoyl ist; —S(O)—CH=$CHNHCOCH_3$ und —S—CH=$CHNHCOCH_3$ sein kann; und R^6



ist, worin R^7 Wasserstoff, Hydroxy oder Hydroxysulfonyloxy ist, oder R^6 Wasserstoff ist, einschließlich aller möglichen stereoisomeren Formen.

3. Verfahren nach Anspruch 1 oder 2, in dem die Bestandteile mit einem pharmazeutischen Träger vermischt werden.

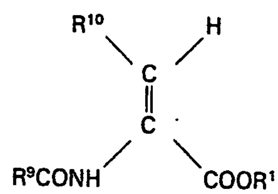
4. Verfahren nach Anspruch 3, in dem der Träger zur Injektion angepaßt ist.

5. Verfahren nach Anspruch 1 oder 2, in dem die Thienamycin-Typ-Verbindung Thienamycin ist.

6. Verfahren nach Anspruch 1 oder 2, in dem die Thienamycin-Typ-Verbindung N-Formimidoylthienamycin ist.

7. Verfahren nach Anspruch 1 oder 2, in dem die Thienamycin-Typ-Verbindung N-Acetimidoylthienamycin ist.

8. Verfahren nach Anspruch 1 oder 2, in dem der Dipeptidaseinhibitor eine Verbindung der folgenden Formel ist.



worin R^9 und R^{10} Kohlenwasserstoffreste im Bereich von 3—10 bzw. 1—15 Kohlenstoffatomen sind; wobei in jeder dieser R^9- oder R^{10}-Kohlenwasserstoffketten 1—6 Wasserstoffe ersetzt sein können durch Halogene oder ein nicht endständiges Methylen ersetzt sein kann durch Sauerstoff oder Schwefel, einschließlich der oxydierten Formen des letzteren; ferner ein endständiger Wasserstoff in R^{10} auch ersetzt sein kann durch eine Hydroxyl- oder Thiolgruppe, die acyliert oder carbamoyliert sein kann; oder der Wasserstoff ersetzt sein kann durch eine Aminogruppe, die in Derivatform vorliegen kann, wie in einer Acylamino-, Ureido-, Amidino-, Guanidino- oder Alkyl- oder substituierten Alkylamino-gruppe, einschließlich quaternärer Stickstoffgruppen; oder alternativ ein Ersatz durch saure Gruppen vorliegen kann, wie Carbonsäure-, Phosphonsäure- oder Sulfonsäuregruppen oder Ester oder Amide davon, sowie Cyano; oder Kombinationen davon, wie eine endständige Aminosäuregruppe; und R^1 Wasserstoff oder Niedrigalkyl(C_{1-6}) oder Dialkylaminoalkyl oder ein pharmazeutisch brauchbares Kation ist.

9. Verfahren nach Anspruch 8, in dem R^9 eine gerade, verzweigte oder Cyclo-alkylgruppe mit 3 bis 10 Kohlenstoffatomen einschließlich Alkylcycloalkyl und Dialkylcycloalkyl ist, vorausgesetzt, daß der zur Carbonylgruppe benachbarte Kohlenstoff nicht tertiär sein kann.

10. Verfahren nach Anspruch 8, in dem R^9 in dem Dipeptidaseinhibitor sein kann R^4, worin R^4 verzweigter oder cyclischer Kohlenwasserstoff mit 3—10 Kohlenstoffatomen ist;

—R^5R^{11}, worin R^5 Cycloalkyl mit 3—6 Kohlenstoffatomen ist und R^{11} entweder 1 oder 2 Alkylsubstituenten ist, die verbunden sein können unter Bildung eines weiteren Ringes an der Cycloalkylgruppe oder R^{11} 1 oder 2 Chlorsubstituenten bedeutet;

—$R^{12}R^8$, worin R^{12} Alkylen mit 1 bis 3 Kohlenstoffatomen ist und R^8 Cycloalkyl mit 3 bis 6 Kohlenstoffatomen ist.

11. Verfahren nach Anspruch 8, in dem R^9 in dem Dipeptidaseinhibitor 2,2-Dimethylcyclopropyl ist.

12. Verfahren nach Anspruch 8, in dem R^9 in dem Dipeptidaseinhibitor 2,2-Dichlorocyclopropyl ist.

13. Verfahren nach Anspruch 8, in dem der Dipeptidaseinhibitor Z-2-(2,2-Dimethylcyclopropancarboxamido)-2-octensäure ist.